(12) STANDARD PATENT (11) Application No. **AU 2014373640 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
VAR2CSA-drug conjugates

(51) International Patent Classification(s)
C07K 14/445 (2006.01) ***C07K 17/00*** (2006.01)
A61P 35/00 (2006.01)

(21) Application No: **2014373640** (22) Date of Filing: **2014.12.29**

(87) WIPO No: **WO15/095952**

(30) Priority Data

(31) Number	(32) Date	(33) Country
62/051,899	**2014.09.17**	**US**
62/051,886	**2014.09.17**	**US**
61/921,242	**2013.12.27**	**US**

(43) Publication Date: **2015.07.02**
(44) Accepted Journal Date: **2018.08.30**

(71) Applicant(s)
VAR2 Pharmaceuticals APS;Zymeworks Inc.

(72) Inventor(s)
Rich, James R.;Babcock, John;El-Salanti, Ali;Daugaard, Mads;Dahlback, Madeleine;Hedberg, Bradley John;WINTERS, Geoffrey C.;MANDEL, Alexander L.;BOURQUE, Elyse Marie Josée;HSIEH, Tom Han Hsiao

(74) Agent / Attorney
Spruson & Ferguson, GPO Box 3898, Sydney, NSW, 2001, AU

(56) Related Art
WO 2013117705 A1
WO 2004026293 A2
WO 2006027711 A2

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
2 July 2015 (02.07.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/095952 A1

(51) International Patent Classification:
C07K 14/445 (2006.01) *A61P 35/00* (2006.01)
A61K 47/48 (2006.01) *C07K 17/00* (2006.01)

(21) International Application Number:
PCT/CA2014/000919

(22) International Filing Date:
29 December 2014 (29.12.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/921,242 27 December 2013 (27.12.2013) US
62/051,886 17 September 2014 (17.09.2014) US
62/051,899 17 September 2014 (17.09.2014) US

(71) Applicants: **THE CENTRE FOR DRUG RESEARCH AND DEVELOPMENT** [CA/CA]; 2405 Wesbrook Mall, Fourth Floor, Vancouver, British Columbia V6T 1Z3 (CA). **CDRD VENTURES INC.** [CA/CA]; 2405 Wesbrook Mall, Fourth Floor, Vancouver, British Columbia V6T 1Z3 (CA). **KAIROS THERAPEUTICS INC.** [CA/CA]; 2405 Wesbrook Mall, Fourth Floor, Vancouver, British Columbia V6T 1Z3 (CA). **VAR2 PHARMACEUTICALS APS** [DK/DK]; c/o Cobis A/S, Ole Maaløes Vej 3, DK-2200 Copenhagen N (DK).

(72) Inventors: **RICH, James, R.**; 1370 18th Ave East, Vancouver, British Columbia V5B 1H5 (CA). **BABCOCK, John**; 4480 West 12th Avenue, Vancouver, British Columbia V6R 2R2 (CA). **EL-SALANTI, Ali**; Smorumvej 50, 2700 Bronshoj (DK). **DAUGAARD, Mads**; 1374 Island Park Walk, Vancouver, British Columbia V6H 3T5 (CA). **DAHLBACK, Madeleine**; Bremadsvägen 88, 23391, Svedala (SE). **HEDBERG, Bradley, John**; 4016 Glen Drive, Vancouver, British Columbia V5V 4T3 (CA).

(74) Agents: **ALEXANDER, Sean** et al.; Gowling Lafleur Henderson LLP, 2300 - 550 Burrard Street, Vancouver, British Columbia V6C 2B5 (CA).

(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, 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, ST, 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))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2015/095952 A1

(54) Title: VAR2CSA-DRUG CONJUGATES

(57) Abstract: VAR2CSA-drug conjugates having biological activity are disclosed. Methods associated with preparation and use of such conjugates, as well as pharmaceutical compositions comprising such conjugates, are also disclosed.

VAR2CSA-DRUG CONJUGATES

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. §119(e) of U.S. Provisional Patent Application No. 61/921,242, U.S. Provisional Patent Application No. 62/051,886, filed September 17, 2014, filed December 27, 2013, and U.S. Provisional Patent Application No. 62/051,899, filed September 17, 2014, which applications are incorporated herein by reference in their entireties.

BACKGROUND

FIELD

The invention relates to drug conjugates, compositions comprising the same, and methods of using such drug conjugates and compositions for the treatment of cancer and other diseases.

DESCRIPTION OF THE RELATED ART

VAR2CSA Protein

Proteoglycans are proteins conjugated to glycosaminoglycan (GAG) chains. These proteins are distributed inside cells, on the cell membrane and in the extracellular matrix serving a variety of functions: cartilage matrix formation; the structural organization of tissues; organizations of basement membranes; regulating the role of secretory vesicles; binding of cytokines, chemokines, growth factors, and morphogens; protease receptors and protease inhibitors; co-receptors, tyrosine-kinase-type growth factor receptors; as endocytic receptors; facilitate cell attachment, cell-cell interactions, and cell motility as well as cell migration.

The malaria parasite *Plasmodium falciparum* utilizes host cell proteoglycans in almost all stages of its complex life cycle. The sporozoite infects hepatocytes in the liver through surface-expressed circumsporozoite protein interacting with highly sulfated heparan sulfate proteoglycans (HSPG). Merozoite infection of the erythrocytes is mediated by EBA-175 binding to sialic acid on glycophorin A. In addition, a number of *Plasmodium falciparum* Erythrocyte Membrane Protein 1 (PfEMP1) proteins, mediating host endothelial adhesion, have been described as glycan-binding. One of these is VAR2CSA, which is a unique member of the PfEMP1 protein family. VAR2CSA binds with high affinity to a distinct form of chondroitin sulfate A (CSA), attached to proteoglycans, so called Chondroitin Sulfate Proteoglycan (CSPG), in the intervillous spaces of the placenta. This type of CSA is referred to as placental-like CSA (plCSA). VAR2CSA is a large multi-domain protein (350 kDa) expressed on the surface of *P. falciparum*-infected erythrocytes (IEs), and the VAR2CSA-plCSA interaction is responsible for placenta specific sequestration in placental malaria (PM).

Importantly, recombinant full-length VAR2CSA ecto-domain from FCR3 and 3D7 type parasites has shown affinity for plCSA in the low nano-molar range.

CSA belongs to the family of glycosaminoglycans (GAGs), which are linear polymers of alternating amino sugars and hexuronic acid residues, attached to proteoglycans. There are several types of GAGs including; chondroitin sulfate (CS), dermatan sulfate (DS or CSB), heparan sulfate (HS) and heparin. While the polysaccharide backbone of these GAGs is simple, considerable diversity arises in modifications such as sulfation and uronate epimerization. This is the basis for the wide variety in domain structure and biological activities of different GAGs.

CS interacts with many important factors such as growth hormones, cytokines, chemokines, and adhesion molecules and is thought to be involved in structural stabilization, cytokinesis, cell proliferation, differentiation, cell migration, tissue morphogenesis, organogenesis, infection, and wound repair. CS chains are composed of alternating units of *N*-acetyl-D-galactosamine (GalNAc) and glucuronic acid residues. Glucuronic acid can be sulfated at its C_2 position and GalNAc can be sulfated at C_4 and/or C_6, giving rise to various disaccharide units. Varying modifications of the sugar backbone allows structural and functional heterogeneity of the CS chains.

Chondroitin sulfate proteoglycan 4 (CSPG4), also known as High Molecular Weight-Melanoma Associated Antigen (HMW-MAA) or melanoma-associated chondroitin sulfate proteoglycan (MSCP), is a cell surface proteoglycan which has been shown to be expressed by melanoma cells. CSPG4/MSCP/HMW-MAA is a large proteoglycan characterized by having CS chains on the protein backbone.

VAR2CSA retains its ability to bind with high affinity and specificity to certain chondroitin sulfate proteoglycans with minimal structural elements of the polypeptide sequence. The core plCSA-binding site lies within the DBL2X domain including small parts of the flanking interdomain regions. The binding does not depend on the ID2b region, or on the DBL1X or DBL3X flanking domains, as previously suggested. The minimal binding region is ID1-DBL2Xb, which binds CSPG with characteristics comparable to that of full-length VAR2CSA. The ID1-DBL2Xb minimal binding region is much smaller than full-length VAR2CSA, having a molecular weight of only 62 kDa. This VAR2CSA fragment and other VAR2CSA polypeptides bind with high and specific affinity to cancer cells and tissues, which binding is suggested to be through a specific interaction with chondroitin sulfate proteoglycans expressed on the surface of the cancer cells or in the surrounding extracellular matrix (Salanti *et al.*, WO2013/117705). Accordingly, this specific and high affinity binding may be used for the targeting of cancer cells or other tissues or cells with high or otherwise expression, such as inappropriate expression, of this particular type of chondroitin sulfate proteoglycan.

In the medical field, there is a need for stable protein-drug conjugates that can release biologically active compounds selectively at desired target locations having high, or otherwise

2014373640 27 Jul 2018

inappropriate, expression of chondroitin sulfate proteoglycans. The present disclosure fulfills these needs and provides further related advantages.

BRIEF SUMMARY

According to a first aspect, the present invention provides a compound of Formula I:

T–L–P

I

wherein:

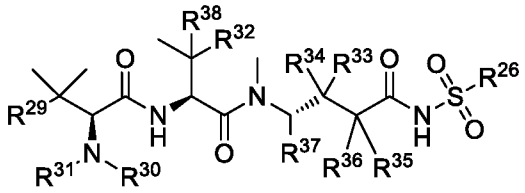
T is a targeting moiety comprising a VAR2CSA polypeptide, the VAR2CSA polypeptide comprising a sequential amino acid sequence of ID1 and DBL2Xb, and

L–P is L^1–P^1 or L^2–P^2;

wherein:

L^1 is a linker, or L^1 is absent;

P^1 is a monovalent radical of a compound of Formula XV:



XV

wherein:

R^{26} is selected from the group consisting of C_1-C_6 alkyl, aryl, aryl-C_1-C_6 alkyl, C_3-C_7 cycloalkyl, heteroaryl, and heterocyclyl, each optionally substituted with one or more substituents selected from: C_1-C_6 alkoxy, C_1-C_6 alkoxycarbonyl, C_1-C_6 alkyl, C_1-C_6 alkylamino, amino, amino-C_1-C_6 alkyl, amino-aryl, amino-C_3-C_7 cycloalkyl, aryl, carboxamide, carboxyl, C_3-C_7 cycloalkyl, cyano, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, halo, hydroxyl, nitro, thio, and thio-C_1-C_6 alkyl;

R^{29} is selected from the group consisting of aryl, C_3-C_7 cycloalkyl, and heteroaryl, each of which is optionally substituted with one or more substituents selected from: C_1-C_4 acylthio, C_2-C_4 alkenyl, C_1-C_4 alkyl, C_1-C_4 alkylamino, C_1-C_4 alkoxy, amino, amino-C_1-C_4 alkyl, halo, C_1-C_4 haloalkyl, hydroxyl, hydroxy-C_1-C_4 alkyl, and thio, wherein C_2-C_4 alkenyl, C_1-C_4

2014373640 27 Jul 2018

alkylamino and C_1-C_4 alkoxy are further optionally substituted with one substituent selected from C_1-C_4 alkylaryl, hydroxyl, and thio;

R^{30} is selected from the group consisting of H and C_{1-6} alkyl;

R^{31} is selected from the group consisting of H and C_{1-6} alkyl;

R^{32} and R^{38} are each independently selected from the group consisting of H, C_{1-6} alkyl and –SH, with the proviso that R^{32} and R^{38} cannot both be H;

R^{33}, R^{34}, R^{35} and R^{36} are each independently H or C_{1-6} alkyl, wherein at least one of R^{33} and R^{34} is H; or R^{34} and R^{35} form a double bond, R^{33} is H, and R^{36} is H or C_{1-6} alkyl; and

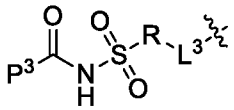
R^{37} is selected from the group consisting of H and C_{1-6} alkyl; and

wherein:

L^2 is a linker;

P^2 is a cytotoxic compound selected from a hemiasterlin, a hemiasterlin analog, a tubulysin, a tubulysin analog, an auristatin, and an auristatin analog; and

L^2–P^2 has the following structure (III):



III

wherein:

R is selected from the group consisting of C_1-C_6 alkyl, aryl, aryl-C_1-C_6 alkyl, C_3-C_7 cycloalkyl, heteroaryl, and heterocyclyl, each optionally substituted with one or more substituents selected from: C_1-C_6 alkoxy, C_1-C_6 alkoxycarbonyl, C_1-C_6 alkyl, C_1-C_6 alkylamino, amino, amino-C_1-C_6 alkyl, amino-aryl, amino-C_3-C_7 cycloalkyl, aryl, carboxamide, carboxyl, C_3-C_7 cycloalkyl, cyano, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, halo, hydroxyl, nitro, thio, and thio-C_1-C_6 alkyl, or R is absent;

P^3 is the remaining portion of compound P^2; and

L^3 is the remaining portion of linker L^2 or is absent.

According to a second aspect, the present invention provides a pharmaceutical composition comprising the compound of the first aspect, and a pharmaceutically acceptable carrier, diluent or excipient.

2014373640 27 Jul 2018

According to a third aspect, the present invention provides a method of treating a disease characterized by abnormal expression of placental-like chondroitin sulfate A (pICSA) in a mammal, comprising administering to a mammal in need thereof an effective amount of the compound of the first aspect, or the pharmaceutical compound of the second.

According to a fourth aspect, the present invention provides use of the compound of the first aspect in the manufacture of a medicament for treating a disease characterized by abnormal expression of placental-like chondroitin sulfate A (p1CSA) in a mammal.

BRIEF SUMMARY

In brief, the present disclosure is directed to biologically active protein-drug conjugates and methods of using such protein-drug conjugates. Provided are protein-drug conjugates which are compounds of Formula I:

T–L–P

I

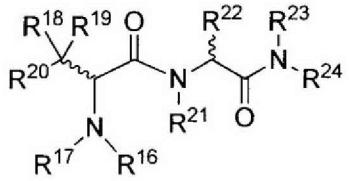
wherein:

T is a targeting moiety comprising a VAR2CSA polypeptide;

L–P is selected from: L^1–P^1 or L^2–P^2;

L^1 is a linker, or L^1 is absent;

P^1 is a monovalent radical of a compound of Formula XIV



XIV

wherein:

R^{16} and R^{17} are independently selected from the group consisting of: H and a saturated or unsaturated moiety having a linear, branched, or non-aromatic cyclic skeleton containing one to ten carbon atoms, and the carbon atoms are optionally substituted with: –OH, –I, –Br, –Cl, –F, –CN, $–CO_2H$, –CHO, –COSH, or $–NO_2$; or R^{17} and R^{20} are fused and form a ring;

2014373640 27 Jul 2018

R^{18} and R^{19} are independently selected from the group consisting of: H, R^{25}, and ArR^{25}–, or R^{18} and R^{19} are joined to form a ring;

R^{20} is selected from the group consisting of: H, R^{25}, ArR^{25}–, and Ar; or R^{20} and R^{17}

R^{21} is selected from the group consisting of: H, R^{25}, and ArR^{25}–;

R^{22} and R^{23} are independently selected from the group consisting of: H, R^{25}, and ArR^{25}–;

R^{24} is: –Y–(CO)$NHSO_2$–R^{26}

R^{25} is defined as a saturated or unsaturated moiety having a linear, branched, or non-aromatic cyclic skeleton containing one to ten carbon atoms, zero to four nitrogen atoms, zero

to four oxygen atoms, and zero to four sulfur atoms, and the carbon atoms are optionally substituted with: =O, =S, OH, $-OR^{28}$, $-O_2CR^{28}$, –SH, $-SR^{28}$, $-SOCR^{28}$, $-NH_2$, $-NHR^{28}$, $-N(R^{28})_2$, $-NHCOR^{28}$, $-NR^{28}COR^{28}$, –I, –Br, –Cl, –F, –CN, $-CO_2H$, $-CO_2R^{28}$, –CHO, $-COR^{28}$, $-CONH_2$, $-CONHR^{28}$, $-CON(R^{28})_2$, –COSH, $-COSR^{28}$, $-NO_2$, $-SO_3H$, $-SOR^{28}$, $-SO_2R^{28}$, wherein R^{28} is a linear, branched or cyclic, one to ten carbon saturated or unsaturated alkyl group;

the ring formed by joining R^{18} and R^{19} is a three to seven member non-aromatic cyclic skeleton within the definition of R^{25},

Y is defined as a moiety selected from the group consisting of: a linear, saturated or unsaturated, one to six carbon alkyl group, optionally substituted with R^{25}, ArR^{25}–, or X; and,

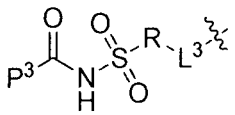
X is defined as a moiety selected from the group consisting of: –OH, $-OR^{25}$, =O, =S, $-O_2CR^{25}$, –SH, $-SR^{25}$, $-SOCR^{25}$, $-NH_2$, $-NHR^{25}$, $-N(R^{25})_2$, $-NHCOR^{25}$, $-NRCOR^{25}$, –I, –Br, –Cl, –F, –CN, $-CO_2H$, $-CO_2R^{25}$, –CHO, $-COR^{25}$, $-CONH_2$, $-CONHR^{25}$, $-CON(R^{25})_2$, –COSH, $-COSR^{25}$, $-NO_2$, $-SO_3H$, $-SOR^{25}$, and $-SO_2R^{25}$;

R^{26} is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, $-COR^{27}$, $-CSR^{27}$, $-OR^{27}$, and $-NHR^{27}$, wherein each R^{27} is, independently, optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, or optionally substituted heteroaryl; and

L^2 is a linker, or L^2 is absent;

P^2 is a biologically active compound; and

L^2–P^2 has the following structure (III):



III

wherein:

R is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, $-COR^{27}$, $-CSR^{27}$, $-OR^{27}$, and $-NHR^{27}$, wherein each R^{27} is, independently, optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, or optionally substituted heteroaryl, or R is absent;

P^3 is the remaining portion of compound P^2; and

L^3 is optionally the remaining portion of linker L^2 when L^2 is present.

In a preferred embodiment, R is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl, or R is absent.

In another embodiment, a method of using a compound of Formula I in therapy is provided. In particular, the present disclosure provides a method of treating cancer in a mammal comprising administering to a mammal in need thereof an effective amount of a compound of Formula I or a pharmaceutical composition comprising a compound of Formula I and a pharmaceutically acceptable carrier diluent or excipient.

In another embodiment, the present disclosure provides a method of inhibiting tumor growth in a mammal comprising administering to a mammal in need thereof an effective amount of a compound of Formula I or a pharmaceutical composition comprising a compound of Formula I and a pharmaceutically acceptable carrier, diluent or excipient.

In another embodiment, the present disclosure provides a method of killing cancer cells *in vitro* using a compound of construct. In another embodiment, the present disclosure provides a method of killing cancer cells *in vivo* in a mammal, comprising administering to a mammal in need thereof an effective amount of a compound of Formula I or a pharmaceutical composition comprising a compound of Formula I and a pharmaceutically acceptable carrier, diluent or excipient.

These and other aspects of the disclosure will be apparent upon reference to the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows summarized cytotoxicity data (EC_{50}) for each of Compounds A-E for two cell lines (HCC1954 and Jurkat)

Figure 2 shows a cytotoxicity data plot for Compound A on two cell lines (HCC1954 and Jurkat)

Figure 3 shows a cytotoxicity data plot for Compound B on two cell lines (HCC1954 and Jurkat).

Figure 4 shows a cytotoxicity data plot for Compound C on two cell lines (HCC1954 and Jurkat).

Figure 5 shows a cytotoxicity data plot for Compound D on two cell lines (HCC1954 and Jurkat).

Figure 6 shows a cytotoxicity data plot for Compound E on two cell lines (HCC1954 and Jurkat).

Figure 7 shows the *in vivo* results of administration of Compound F, Compound 14, or Compound 23 on tumor volume in female athymic nude mice with established tumors.

Figure 8: shows the SEC-UPLC-QTof-MS MaxEnt1 processed intact mass of VAR2-Compound O. The MS signals at 115323 Da, 117662 Da and 119999 Da are consistent with conjugation of 1, 3 and 5 toxins, with a mean conjugation level of ~4 toxins per protein.

Figure 9 shows the: SEC-UPLC-QTof-MS MaxEnt1 processed intact mass of VAR2-Compound KK. The profile of the deconvolved MS data is consistent with conjugation of up to 5 toxins (Compound KK), but with a mean drug load of ~2.5 drugs.

Figure 10 shows the analysis of free unconjugated toxin-linker in VAR2CSA drug conjugate preparation. X-axis: Time. Y-axis: Intensity. Lines (based on decreasing height of apex) correspond to 20 nM drug-linker, 10 nM drug-linker, 1 nM drug linker, 0.5 nM drug linker, and analyte.

Figure 11 shows the specificity of non-modified DBL1-ID2a (MP1255) binding to Myla2059 cell line in the presence and absence of CSA (Sigma C9819).

Figure 12 shows the specificity of VAR2-Compound O (Cysteine conjugate) binding to the Myla2059 cell line in the presence and absence of CSA (Sigma C9819).

Figure 13 shows the specificity of VAR2-Compound KK (Lysine conjugate) binding to the Myla2059 cell line in the presence and absence of CSA (Sigma C9819).

Figure 14 shows the cytotoxicity of VAR2CSA drug conjugate against Colo205 cells. The VAR2 protein used was DBL1-ID2a. VAR2-Compound O – circles. VAR2-Compound O with added CSA (Sigma C9819) – diamonds. CSA alone – squares.

Figure 15 shows the cytotoxicity of VAR2CSA drug conjugates against Colo205 cells. VAR2-Compound O prepared by cysteine conjugation – triangles. VAR2-Compound KK prepared by lysine conjugation – circles.

Figure 16 shows the cytotoxicity of VAR2CSA drug conjugates against Colo205 cells. VAR2-Compound O – circles. Toxin-linker alone – squares.

Figure 17 shows the cytotoxicity of VAR2CSA drug conjugates against UM-UC 3 Cells. VAR2-Compound O – circles. VAR2CSA alone – diamonds.

Figure 18 shows the cytotoxicity of VAR2CSA drug conjugates against U138 MG Cells. VAR2-Compound O – circles. VAR2CSA alone – diamonds.

Figure 19 shows the cytotoxicity of VAR2CSA drug conjugates against OVCAR-3 Cells. VAR2-Compound O – circles. VAR2CSA alone – diamonds.

Figure 20 shows the body weights of mice in a tolerability study with VAR2-Compound O.

Figure 21 shows the body weights of mice in a Karpas 299 xenograft efficacy study following three IV doses of test articles.

Figure 22 shows tumor volumes of mice in a Karpas 299 xenograft efficacy study following three IV doses of test articles.

Figure 23 shows the body weights of mice in a PC3 prostate cancer efficacy study.

Figure 24 shows tumor volumes of mice in a PC3 prostate cancer efficacy study.

DETAILED DESCRIPTION

In the following description, certain specific details are set forth in order to provide a thorough understanding of various embodiments of the disclosure. However, one skilled in the art will understand that the disclosure may be practiced without these details.

DEFINITIONS

Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings. When trade names are used herein, applicants intend to independently include the trade name product formulation, the generic drug, and the active pharmaceutical ingredient(s) of the trade name product.

Unless the context requires otherwise, throughout the present specification and claims, the word "comprise" and variations thereof, such as, "comprises" and "comprising" are to be construed in an open, inclusive sense, that is as "including, but not limited to".

Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present disclosure. Thus, the appearances of the phrases "in one embodiment" or "in an embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment. It is appreciated that certain features described herein, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features described herein, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination.

Chemical Groups

All combinations of the embodiments pertaining to the chemical groups represented by the variables (*e.g.*, R^1-R^{50}, Q, X, Y, and Z) contained within the generic chemical formulae described herein, (*e.g.*, II, XIV, XV, and XX) are specifically embraced by the present invention just as if each and every combination was individually explicitly recited, to the extent that such combinations embrace compounds that result in stable compounds (*i.e.*, compounds that can be isolated, characterized and tested for biological activity). In addition, all subcombinations of the chemical groups listed in the embodiments describing such variables, as well as all subcombinations of uses and medical indications described herein, are also specifically embraced by the present invention just

as if each and every subcombination of chemical groups and subcombination of uses and medical indications was individually and explicitly recited herein. In addition, in the event that a list of substituents is listed for any particular variable (*e.g.*, R^1-R^{50}, Q, X, Y, and Z) in a particular embodiment and/or claim, it is understood that each individual substituent may be deleted from the particular embodiment and/or claim and that the remaining list of substituents will be considered to be within the scope of the present disclosure.

The term "acyloxy", as used herein, includes –OC(O)–alkyl, wherein alkyl is as defined herein. Examples of acyloxy include, but are not limited to: formyloxy, acetoxy, propionyloxy, isobutyryloxy, pivaloyloxy, and the like.

The term "acylthio", as used herein, refers to –SC(O)–alkyl, wherein alkyl is as defined herein. Examples of acylthio include, but are not limited to: formylthio, acetylthio, propionylthio, isobutyrylthio, pivaloylthio, and the like.

The term "alkoxycarbonyl", as used herein, refers to –C(O)O–alkyl. Examples of alkoxycarbonyl include, but are not limited to: methoxycarbonyl, ethoxycarbonyl, n-propoxycarbonyl, isopropoxycarbonyl, n-butoxycarbonyl, sec-butoxycarbonyl, isobutoxycarbonyl, t-butoxycarbonyl, pentyloxycarbonyl, isopentyloxycarbonyl, t-pentyloxycarbonyl, neo-pentyloxycarbonyl, 1-methylbutoxycarbonyl, 2-methylbutoxycarbonyl, n-hexyloxycarbonyl, and the like.

"Alkyl" refers to a straight or branched hydrocarbon chain substituent consisting solely of carbon and hydrogen atoms, which is saturated or unsaturated (*i.e.*, contains one or more double and/or triple bonds), having from one to twelve carbon atoms (C_1-C_{12} alkyl), preferably one to eight carbon atoms (C_1-C_8 alkyl) or one to six carbon atoms (C_1-C_6 alkyl), and which is attached to the rest of the molecule by a single bond, *e.g.*, methyl, ethyl, *n*-propyl, 1-methylethyl (*iso*-propyl), *n*-butyl, *n*-pentyl, 1,1-dimethylethyl (*t*-butyl), 3-methylhexyl, 2-methylhexyl, ethenyl, prop-1-enyl, but-1-enyl, pent-1-enyl, penta-1,4-dienyl, ethynyl, propynyl, butynyl, pentynyl, hexynyl, and the like. Unless stated otherwise specifically in the specification, an alkyl group may be optionally substituted.

"Alkylene" or "alkylene chain" or "alkyldiyl" refers to a straight or branched divalent hydrocarbon chain linking the rest of the molecule to a substituent group, consisting solely of carbon and hydrogen, which is saturated or unsaturated (*i.e.*, contains one or more double and/or triple bonds), and having from one to twelve carbon atoms, *e.g.*, methylene, ethylene, propylene, *n*-butylene, ethenylene, propenylene, *n*-butenylene, propynylene, *n*-butynylene, and the like. The alkylene chain is attached to the rest of the molecule through a single or double bond and to the substituent group through a single or double bond. The points of attachment of the alkylene chain to the rest of the molecule and to the substituent group can be through one carbon or any two carbons within the chain. Unless stated otherwise specifically in the specification, an alkylene chain may be optionally substituted.

The term "alkenyldiyl", as used herein, refers to a straight or branched unsaturated hydrocarbon di-radical containing the specified number of carbon atoms, and one or more carbon-carbon double bonds, *e.g.*, C_2-C_6 alkenyldiyl, C_2-C_4 alkenyldiyl, or C_2 alkenyldiyl. Examples of alkenyldiyl include, but are not limited to: ethenyldiyl, n-propenyldiyl, isopropenyldiyl, n-butenyldiyl, sec-butenyldiyl, isobutenyldiyl, t-butenyldiyl, pentenyldiyl, isopentenyldiyl, t-pentenyldiyl, neo-pentenyldiyl, 1-methylbutenyldiyl, 2-methylbutenyldiyl, n-hexenyldiyl, and the like.

"Alkoxy" refers to a substituent of the formula $-OR_a$ where R_a is an alkyl substituent as defined above containing one to twelve carbon atoms. Unless stated otherwise specifically in the specification, an alkoxy group may be optionally substituted.

"Alkylamino" refers to a substituent of the formula $-NHR_a$ or $-NR_aR_a$ where each R_a is, independently, an alkyl substituent as defined above containing one to twelve carbon atoms. Unless stated otherwise specifically in the specification, an alkylamino group may be optionally substituted.

"Amino" refers to the $-NH_2$ substituent.

The term "amino-cycloalkyl", as used herein, refers to a cycloalkyl group, substituted with one amino substituent, as those terms are defined herein. Examples of amino-cycloalkyl include, but are not limited to: aminocyclopropyl, aminocyclobutyl, aminocyclopentyl, aminocyclohexyl, and the like.

The term "amino-alkyl", as used herein, refers to an alkyl group, substituted with one amino substituent, as those terms are defined herein. Examples of amino-alkyl include, but are not limited to: aminomethyl, aminoethyl, amino-n-propyl, amino-isopropyl, amino-n-butyl, amino-sec-butyl, amino-isobutyl, amino-t-butyl, amino-pentyl, amino-isopentyl, amino-t-pentyl, amino-neo-pentyl, amino-1-methylbutyl, amino-2-methylbutyl, amino-n-hexyl, and the like.

The term "amino-aryl", as used herein, refers to an aryl group, substituted with one amino substituent, as those terms are defined herein. Examples of amino-aryl include, but are not limited to: amino-phenyl, amino-naphthalenyl, and the like.

"Aryl" refers to a hydrocarbon ring system substituent comprising hydrogen, 6 to 18 carbon atoms and at least one aromatic ring. For purposes of this disclosure, the aryl substituent may be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which may include fused or bridged ring systems. Aryl substituents include, but are not limited to, aryl substituents derived from aceanthrylene, acenaphthylene, acephenanthrylene, anthracene, azulene, benzene, chrysene, fluoranthene, fluorene, *as*-indacene, *s*-indacene, indane, indene, naphthalene, phenalene, phenanthrene, pleiadene, pyrene, and triphenylene. Unless stated otherwise specifically in the specification, the term "aryl" or the prefix "ar-" (such as in "aralkyl") is meant to include aryl substituents that are optionally substituted.

"Aralkyl" refers to a substituent of the formula $-R_b-R_c$ where R_b is an alkylene chain as defined above and R_c is one or more aryl substituents as defined above, for example, benzyl,

diphenylmethyl and the like. Unless stated otherwise specifically in the specification, an aralkyl group may be optionally substituted.

The term "carboxamide", as used herein, refers to $-C(O)NH_2$.

The term "carboxyl", as used herein, refers to $-C(O)OH$.

"Cyano" refers to the $-CN$ substituent.

"Cycloalkyl" or "carbocyclic ring" refers to a stable non-aromatic monocyclic or polycyclic hydrocarbon substituent consisting solely of carbon and hydrogen atoms, which may include fused or bridged ring systems, having from three to fifteen carbon atoms, preferably having from three to ten carbon atoms, and which is saturated or unsaturated and attached to the rest of the molecule by a single bond. Monocyclic substituents include, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. Polycyclic substituents include, for example, adamantyl, norbornyl, decalinyl, 7,7-dimethyl-bicyclo[2.2.1]heptanyl, and the like. Unless otherwise stated specifically in the specification, a cycloalkyl group may be optionally substituted.

"Cycloalkylalkyl" refers to a substituent of the formula $-R_bR_d$ where R_d is an alkylene chain as defined above and R_g is a cycloalkyl substituent as defined above. Unless stated otherwise specifically in the specification, a cycloalkylalkyl group may be optionally substituted.

"Fused" refers to any ring structure described herein which is fused to an existing ring structure in the compounds of the disclosure. When the fused ring is a heterocyclyl ring or a heteroaryl ring, any carbon atom on the existing ring structure which becomes part of the fused heterocyclyl ring or the fused heteroaryl ring may be replaced with a nitrogen atom.

"Halo" or "halogen" refers to bromo, chloro, fluoro or iodo.

"Haloalkyl" refers to an alkyl substituent, as defined above, that is substituted by one or more halo substituents, as defined above, *e.g.*, trifluoromethyl, difluoromethyl, trichloromethyl, 2,2,2-trifluoroethyl, 1,2-difluoroethyl, 3-bromo-2-fluoropropyl, 1,2-dibromoethyl, and the like. Unless stated otherwise specifically in the specification, a haloalkyl group may be optionally substituted.

The term "haloalkoxy", as used herein, refers to $-O-$haloalkyl, wherein haloalkyl is as defined herein. Examples of haloalkoxy include, but are not limited to: difluoromethoxy, trifluoromethoxy, 2,2,2-trifluoroethoxy, pentafluoroethoxy, and the like.

"Heteroaryl" refers to a 5- to 14-membered ring system substituent comprising hydrogen atoms, one to thirteen carbon atoms, one to six heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, and at least one aromatic ring. For purposes of this disclosure, the heteroaryl substituent may be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which may include fused or bridged ring systems; and the nitrogen, carbon or sulfur atoms in the heteroaryl substituent may be optionally oxidized; the nitrogen atom may be optionally quaternized. Examples include, but are not limited to, azepinyl, acridinyl, benzimidazolyl, benzothiazolyl, benzindolyl, benzodioxolyl, benzofuranyl, benzooxazolyl, benzothiazolyl, benzothiadiazolyl,

benzo[*b*][1,4]dioxepinyl, 1,4-benzodioxanyl, benzonaphthofuranyl, benzoxazolyl, benzodioxolyl, benzodioxinyl, benzopyranyl, benzopyranonyl, benzofuranyl, benzofuranonyl, benzothienyl (benzothiophenyl), benzotriazolyl, benzo[4,6]imidazo[1,2-a]pyridinyl, carbazolyl, cinnolinyl, dibenzofuranyl, dibenzothiophenyl, furanyl, furanonyl, isothiazolyl, imidazolyl, indazolyl, indolyl, indazolyl, isoindolyl, indolinyl, isoindolinyl, isoquinolyl, indolizinyl, isoxazolyl, naphthyridinyl, oxadiazolyl, 2-oxoazepinyl, oxazolyl, oxiranyl, 1-oxidopyridinyl, 1-oxidopyrimidinyl, 1-oxidopyrazinyl, 1-oxidopyridazinyl, 1-phenyl-1*H*-pyrrolyl, phenazinyl, phenothiazinyl, phenoxazinyl, phthalazinyl, pteridinyl, purinyl, pyrrolyl, pyrazolyl, pyridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, quinazolinyl, quinoxalinyl, quinolinyl, quinuclidinyl, isoquinolinyl, tetrahydroquinolinyl, thiazolyl, thiadiazolyl, triazolyl, tetrazolyl, triazinyl, and thiophenyl (i.e. thienyl). Unless stated otherwise specifically in the specification, a heteroaryl group may be optionally substituted.

"*N*-heteroaryl" refers to a heteroaryl substituent as defined above containing at least one nitrogen and where the point of attachment of the heteroaryl substituent to the rest of the molecule is through a nitrogen atom in the heteroaryl substituent. Unless stated otherwise specifically in the specification, an *N*-heteroaryl group may be optionally substituted.

"Heteroarylalkyl" refers to a substituent of the formula $-R_bR_f$ where R_b is an alkylene chain as defined above and R_f is a heteroaryl substituent as defined above. Unless stated otherwise specifically in the specification, a heteroarylalkyl group may be optionally substituted.

The term "heteroaryldiyl", as used herein, refers to a divalent radical derived from a 6- to 12-membered mono- or bicyclic ring system wherein at least one ring atom is a heteroatom and at least one ring is aromatic. Examples of a heteroatom include, but are not limited to: O, S, N, and the like. Examples of heteroaryldiyl include, but are not limited to: thiazolyldiyl, 2,4-thiazolyldiyl, triazolyldiyl, 1,2,3-triazolyl-1,4-diyl, pyridyldiyl, benzofuranyldiyl, pyrazinyldiyl, pyridazinyldiyl, pyrimidinyldiyl, triazinyldiyl, quinolinyldiyl, benzoxazolyldiyl, benzothiazolyldiyl, 1*H*-benzimidazolyldiyl, isoquinolinyldiyl, quinazolinyldiyl, quinoxalinyldiyl, pyrrolyldiyl, indolyldiyl, 1*H*-benzoimidazol-2-yldiyl, benzo[1,3]dioxol-5-yldiyl, 3,4-dihydro-2*H*-benzo[1,4]oxazin-7-yldiyl, 2,3-dihydro-benzofurn-7-yldiyl, 2,3-dihydro-indol-1-yldiyl, and the like. Examples of include, but are not limited to:, and the like.

"Heterocyclyl" or "heterocyclic ring" refers to a stable 3- to 18-membered non-aromatic ring substituent which consists of two to twelve carbon atoms and from one to six heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur. Unless stated otherwise specifically in the specification, the heterocyclyl substituent may be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which may include fused or bridged ring systems; and the nitrogen, carbon or sulfur atoms in the heterocyclyl substituent may be optionally oxidized; the nitrogen atom may be optionally quaternized; and the heterocyclyl substituent may be partially or fully saturated. Examples of such heterocyclyl substituents include, but are not limited to, dioxolanyl, thienyl[1,3]dithianyl,

decahydroisoquinolyl, imidazolinyl, imidazolidinyl, isothiazolidinyl, isoxazolidinyl, morpholinyl, octahydroindolyl, octahydroisoindolyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolidinyl, oxazolidinyl, piperidinyl, piperazinyl, 4-piperidonyl, pyrrolidinyl, pyrazolidinyl, quinuclidinyl, thiazolidinyl, tetrahydrofuryl, trithianyl, tetrahydropyranyl, thiomorpholinyl, thiamorpholinyl, 1-oxo-thiomorpholinyl, and 1,1-dioxo-thiomorpholinyl. Unless stated otherwise specifically in the specification, a heterocyclyl group may be optionally substituted.

"*N*-heterocyclyl" refers to a heterocyclyl substituent as defined above containing at least one nitrogen and where the point of attachment of the heterocyclyl substituent to the rest of the molecule is through a nitrogen atom in the heterocyclyl substituent. Unless stated otherwise specifically in the specification, a *N*-heterocyclyl group may be optionally substituted.

"Heterocyclylalkyl" refers to a substituent of the formula $-R_bR_e$ where R_b is an alkylene chain as defined above and R_e is a heterocyclyl substituent as defined above, and if the heterocyclyl is a nitrogen-containing heterocyclyl, the heterocyclyl may be attached to the alkyl substituent at the nitrogen atom. Unless stated otherwise specifically in the specification, a heterocyclylalkyl group may be optionally substituted.

The term "heterocyclyldiyl", as used herein, refers to a divalent radical derived from a 3- to 12-membered mono- or bicyclic non-aromatic ring system wherein at least one ring atom is a heteroatom. Examples of a heteroatom include, but are not limited to: O, S, N, and the like. A heterocyclyldiyl substituent can be attached via any two of its available ring atoms, for example, ring carbons, or ring nitrogens. In some embodiments, the heterocyclyldiyl is a 3-, 4-, 5-, 6- or 7-membered containing ring. Examples of a heterocyclyldiyl group include, but are not limited to: aziridin-1-yldiyl, aziridin-2-yldiyl, azetidin-1-yldiyl, azetidin-2-yldiyl, azetidin-3-yldiyl, piperidin-1-yldiyl, piperidin-2-yldiyl, piperidin-3-yldiyl, piperidin-4-yldiyl, morpholin-2-yldiyl, morpholin-3-yldiyl, morpholin-4-yldiyl, piperazin-1-yldiyl, piperazin-2-yldiyl, piperazin-3-yldiyl, piperazin-4-yldiyl, pyrrolidin-1-yldiyl, pyrrolidin-2-yldiyl, pyrrolidin-3-yldiyl, [1,3]-dioxolan-2-yldiyl, thiomorpholin-4-yldiyl, [1,4]oxazepan-4-yldiyl, 1,1-dioxo-1λ6-thiomorpholin-4-yldiyl, azepan-1-yldiyl, azepan-2-yldiyl, azepan-3-yldiyl, azepan-4-yldiyl, octahydro-quinolin-1-yldiyl, octahydro-isoquinolin-2-yldiyl, and the like.

"Hydroxy" or "hydroxyl" refers to the –OH substituent.

The term "hydroxy-alkyl", as used herein, refers to an alkyl group, substituted with one hydroxy substituent, as those terms are defined herein. Examples of hydroxy-alkyl include, but are not limited to: hydroxymethyl, hydroxyethyl, hydroxy-n-propyl, hydroxy-isopropyl, hydroxy-n-butyl, hydroxy-sec-butyl, hydroxy-isobutyl, hydroxy-t-butyl, hydroxy-pentyl, hydroxy-isopentyl, hydroxy-t-pentyl, hydroxy-neo-pentyl, hydroxy-1-methylbutyl, hydroxy-2-methylbutyl, hydroxy-n-hexyl, and the like.

"Imino" refers to the =NH substituent.

"Thioalkyl" refers to a substituent of the formula $-SR_a$ where R_a is an alkyl substituent as defined above containing one to twelve carbon atoms. Unless stated otherwise specifically in the specification, a thioalkyl group may be optionally substituted.

"Nitro" refers to the $-NO_2$ substituent.

"Oxo" refers to the =O substituent.

"Thiol" refers to the –SH substituent.

"Thioxo" refers to the =S substituent.

The term "substituted" used herein means any of the above groups (*i.e.,* alkyl, alkylene, alkoxy, alkylamino, thioalkyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl, haloalkyl, heterocyclyl, *N*-heterocyclyl, heterocyclylalkyl, heteroaryl, *N*-heteroaryl and/or heteroarylalkyl) wherein at least one hydrogen atom is replaced by a bond to a non-hydrogen atoms such as, but not limited to: a halogen atom such as F, Cl, Br, and I; an oxygen atom in groups such as hydroxyl groups, alkoxy groups, and ester groups; a sulfur atom in groups such as thiol groups, thioalkyl groups, sulfone groups, sulfonyl groups, and sulfoxide groups; a nitrogen atom in groups such as azides, amines, amides, alkylamines, dialkylamines, arylamines, alkylarylamines, diarylamines, N-oxides, imides, and enamines; a silicon atom in groups such as trialkylsilyl groups, dialkylarylsilyl groups, alkyldiarylsilyl groups, and triarylsilyl groups; and other heteroatoms in various other groups. "Substituted" also means any of the above groups in which one or more hydrogen atoms are replaced by a higher-order bond (e.g., a double- or triple-bond) to a heteroatom such as oxygen in oxo, carbonyl, carboxyl, and ester groups; and nitrogen in groups such as imines, oximes, hydrazones, and nitriles. For example, "substituted" includes any of the above groups in which one or more hydrogen atoms are replaced with $-NR_gR_h$, $-NR_gC(=O)R_h$, $-NR_gC(=O)NR_gR_h$, $-NR_gC(=O)OR_h$, $-NR_gC(=NR_g)NR_gR_h$, $-NR_gSO_2R_h$, $-OC(=O)NR_gR_h$, $-OR_g$, $-SR_g$, $-SOR_g$, $-SO_2R_g$, $-OSO_2R_g$, $-SO_2OR_g$, $=NSO_2R_g$, and $-SO_2NR_gR_h$. "Substituted also means any of the above groups in which one or more hydrogen atoms are replaced with $-C(=O)R_g$, $-C(=O)OR_g$, $-C(=O)NR_gR_h$, $-CH_2SO_2R_g$, $-CH_2SO_2NR_gR_h$. In the foregoing, R_g and R_h are the same or different and independently hydrogen, alkyl, alkoxy, alkylamino, thioalkyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl, haloalkyl, heterocyclyl, *N*-heterocyclyl, heterocyclylalkyl, heteroaryl, *N*-heteroaryl and/or heteroarylalkyl. "Substituted" further means any of the above groups in which one or more hydrogen atoms are replaced by a bond to an amino, cyano, hydroxyl, imino, nitro, oxo, thioxo, halo, alkyl, alkoxy, alkylamino, thioalkyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl, haloalkyl, heterocyclyl, *N*-heterocyclyl, heterocyclylalkyl, heteroaryl, *N*-heteroaryl and/or heteroarylalkyl group. In addition, each of the foregoing substituents may also be optionally substituted with one or more of the above substituents.

The present disclosure also meant to encompass all pharmaceutically acceptable compounds of Formula I being isotopically-labelled by having one or more atoms replaced by an atom having a different atomic mass or mass number. Examples of isotopes that can be incorporated into the

disclosed compounds include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine, chlorine, and iodine, such as ^{2}H, ^{3}H, ^{11}C, ^{13}C, ^{14}C, ^{13}N, ^{15}N, ^{15}O, ^{17}O, ^{18}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F, ^{36}Cl, ^{123}I, and ^{125}I, respectively. These radiolabelled compounds are useful to help determine or measure the effectiveness of the compounds, by characterizing, for example, the site or mode of action, or binding affinity to pharmacologically important site of action. Certain isotopically-labelled compounds described herein, for example, those incorporating a radioactive isotope, are useful in drug and/or substrate tissue distribution studies. The radioactive isotopes tritium, *i.e.* ^{3}H, and carbon-14, *i.e.* ^{14}C, are particularly useful for this purpose in view of their ease of incorporation and ready means of detection.

Substitution with heavier isotopes such as deuterium, *i.e.* ^{2}H, may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased *in vivo* half-life or reduced dosage requirements, and hence may be preferred in some circumstances.

Substitution with positron emitting isotopes, such as ^{11}C, ^{18}F, ^{15}O and ^{13}N, can be useful in Positron Emission Topography (PET) studies for examining substrate receptor occupancy. Isotopically-labeled compounds described herein can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described in the Preparations and Examples as set out below using an appropriate isotopically-labeled reagent in place of the non-labeled reagent previously employed.

The present disclosure is also meant to encompass the *in vivo* metabolic products of the disclosed compounds. Such products may result from, for example, the oxidation, reduction, hydrolysis, amidation, esterification, and the like of the administered compound, primarily due to enzymatic processes. Accordingly, the present disclosure includes compounds produced by a process comprising administering a compound of this disclosure to a mammal for a period of time sufficient to yield a metabolic product thereof. Such products are typically identified by administering a radiolabelled compound of the disclosure in a detectable dose to an animal, such as rat, mouse, guinea pig, monkey, or to human, allowing sufficient time for metabolism to occur, and isolating its conversion products from the urine, blood or other biological samples.

The compounds of the disclosure, or their pharmaceutically acceptable salts may contain one or more asymmetric centers and may thus give rise to enantiomers, diastereomers, and other stereoisomeric forms that may be defined, in terms of absolute stereochemistry, as (*R*)- or (*S*)- or, as (D)- or (L)- for amino acids. The present disclosure is meant to include all such possible isomers, as well as their racemic and optically pure forms. Optically active (+) and (-), (*R*)- and (*S*)-, or (D)- and (L)- isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques, for example, chromatography and fractional crystallization. Conventional techniques for the preparation/isolation of individual enantiomers include chiral synthesis from a suitable optically pure precursor or resolution of the racemate (or the racemate of a salt or derivative) using, for

example, chiral high pressure liquid chromatography (HPLC). When the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both *E* and *Z* geometric isomers. Likewise, all tautomeric forms are also intended to be included.

Other Definitions

The term "amino acid" or "amino acid residue" as used herein includes any one of the twenty naturally occurring amino acids, the D-form of any one of the naturally occurring amino acids, non-naturally occurring amino acids, and derivatives, analogs, and mimetics thereof. Any amino acid, including naturally occurring amino acids, may be purchased commercially or synthesized by methods known in the art. Examples of non-naturally-occurring amino acids include citrulline ("Cit"), norleucine ("Nle"), norvaline ("Nva"), p-Alanine, L-or D-naphthalanine, ornithine ("Orn"), homoarginine (homoArg) and others well known in the peptide art, including those described in M. Bodanzsky, "Principles of Peptide Synthesis," 1st and 2nd revised ed., Springer-Verlag, New York, N.Y., 1984 and 1993, and Stewart and Young, "Solid Phase Peptide Synthesis," 2nd ed., Pierce Chemical Co., Rockford, Ill., 1984. Common amino acids may be referred to by their full name, standard single-letter notation, or standard three-letter notation for example: A, Ala, alanine; C, Cys, cysteine; D, Asp, aspartic; E, Glu, glutamic acid; F, Phe, phenylalanine; G, Gly, glycine; H, His, histidine; I, Ile isoleucine; K, Lys, lysine; L, Leu, leucine; M, Met, methionine; N, Asn, asparagine; P, Pro, proline; Q, Gln, glutamine; R, Arg, arginine; S, Ser, serine; T, Thr, threonine; V, Val, valine; W, Trp, tryptophan; X, Hyp, hydroxyproline; Y, Tyr, tyrosine. Any and all of the amino acids in the compositions herein can be naturally occurring, synthetic, and derivatives or mimetics thereof. When the amino acid residues contain one or more chiral centers, any of the D, L, meso, threo or erythro (as appropriate) racemates or mixtures thereof, fall within the scope of this invention.

The term "another amino acid" as used herein means one amino acid that is different from that amino acid naturally present at that position. This includes but is not limited to amino acids that can be encoded by a polynucleotide. In some embodiments the different amino acid is in natural L-form and can be encoded by a polynucleotide.

The term "construct" is intended to indicate a polynucleotide segment which may be based on a complete or partial naturally occurring nucleotide sequence encoding the polypeptide of interest. The construct may optionally contain other polynucleotide segments. In a similar way, the term "amino acids which can be encoded by polynucleotide constructs" covers amino acids which can be encoded by the polynucleotide constructs defined above, *i.e.* amino acids such as Ala, Val, Leu, Ile, Met, Phe, Trp, Pro, Gly, Ser, Thr, Cys, Tyr, Asn, Glu, Lys, Arg, His, Asp and Gln.

The term "DBL2Xb" as used herein refers to a domain of VAR2CSA characterized by having an amino acid sequence with at least 70% sequence identity to an amino acid sequence identified by 153-577 of SEQ ID NO:1.

The term "derivative" as used herein, is intended to designate a VAR2CSA polypeptide exhibiting substantially the same or improved biological activity relative to wild-type VAR2CSA identified by SEQ ID NO:55 or SEQ ID NO:56, or a fragment thereof, in which one or more of the amino acids of the parent peptide have been chemically modified, *e.g.* by alkylation, PEGylation, acylation, ester formation or amide formation or the like.

A "disease or condition of interest" includes diseases and conditions involving expression, such as inappropriate expression of CSA, such as in cancer, arthritis, arthrosis, multiple sclerosis, healing after neural damage, cartilage repair, wound healing, and in psoriasis.

"Effective amount" or "therapeutically effective amount" refers to that amount of a compound described herein which, when administered to a mammal, preferably a human, is sufficient to effect treatment, as defined below, of the particular indication (*e.g.*, cancer or tumor cells in the mammal, preferably a human). The amount of a compound described herein which constitutes a "therapeutically effective amount" will vary depending on the compound, the condition and its severity, the manner of administration, and the age of the mammal to be treated, but can be determined routinely by one of ordinary skill in the art having regard to his own knowledge and to this disclosure.

The phrases "functional variant", "functional fragment", and "functional derivatives" as used herein refers to variants, fragments, truncated versions, as well as derivatives of SEQ ID NO:55 or SEQ ID NO:56, such as any one of SEQ ID NO:1, 3-5, 10, 11, 29, 34, 36-38, 41, 43-45, 48, 53-56, which polypeptides comprises essential binding sequence parts of SEQ ID NO:55 or SEQ ID NO:56 and at least possess the ability to bind plCSA. Accordingly, such polypeptides are VAR2CSA polypeptides, as used herein. It is to be understood that a VAR2CSA functional variant or functional fragment may have two or three features selected from being a both a variant, and/or a fragment and/or a derivative. A functional variant or fragment of a VAR2CSA polypeptide encompass those that exhibit at least about 25%, such as at least about 50%, such as at least about 75%, such as at least about 90% of the binding affinity of wild-type VAR2CSA polypeptide that has been produced in the same cell type, when tested in the assays as described herein or in WO 2013/117705.

The term "immunologic fragment" as used herein refers to fragment of an amino acid sequence that possesses essentially the same functional activities and the same spatial orientation to be recognized by a targeting moiety. Accordingly a specific targeting moiety will bind both the polypeptide and immunologic fragments thereof.

The term "intracellular metabolite" refers to a compound resulting from a metabolic process or reaction inside a cell on a compound described herein (*e.g.*, a VAR2CSA-drug conjugate). The

metabolic process or reaction may be an enzymatic process such as proteolytic cleavage of a peptide linker of the subject compound, or hydrolysis of a functional group such as a hydrazone, ester, or amide within the subject compound, or degradation of a portion or all of a targeting moiety. In the context of conjugates, intracellular metabolites may include, but are not limited to, VAR2CSA polypeptides and free drug, which may have been separated intracellularly, *i.e.*, after entry, diffusion, uptake or transport into a cell (*e.g.*, by enzymatic cleavage of a conjugate by an intracellular enzyme, or degradation of VAR2CSA polypeptide).

In the context of conjugates, the terms "intracellularly cleaved" and "intracellular cleavage" refer to metabolic processes or reactions inside a cell on a compound described herein whereby the covalent attachment, *e.g.*, the linker between the payload and the targeting moiety is broken, resulting in the free drug dissociated from targeting moiety inside the cell. In some embodiments, the cleaved moieties of the subject compounds are intracellular metabolites. Accordingly, in one embodiment, the invention provides compounds that are cleavage products of a compound of Formula I, which cleavage products include compounds of Formula II. Alternatively, drug may be liberated through the degradation or proteolysis of VAR2CSA polypeptide.

The term "extracellular cleavage" refers a metabolic process or reaction outside a cell on a compound described herein whereby the covalent attachment, *e.g.*, the linker between the payload and the targeting moiety is broken, resulting in the free drug dissociated from the targeting moiety outside the cell. In some embodiments, the cleaved moieties of the subject compounds are initially extracellular metabolites, which may move intracellularly by diffusion and cell permeability or transport.

The term "isolated polypeptide" refers to a polypeptide described herein that (1) has been separated from at least about 50 percent of polynucleotides, lipids, carbohydrates or other materials (*i.e.*, contaminants) with which it is naturally associated (not including post-translational modifications). Preferably, the isolated polypeptide is substantially free from any other contaminating polypeptides or other contaminants that are found in its natural environment, which would interfere with its therapeutic, diagnostic, prophylactic or research use.

The term "ID1" as used herein refers to a domain of VAR2CSA characterized by having an amino acid sequence with at least 70% sequence identity to an amino acid sequence identified by 1-152 of SEQ ID NO:1.

The term "ID2a" as used herein refers to a domain of VAR2CSA characterized by having an amino acid sequence of at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49, at least 50, at least 51, at least 52, at least 53, at least 54, at least 55, at least 56, at least 57, at least 58, at least 59, at least 60, at least 61, or at

least 62, such as the 63 consecutive amino acids from the *N*-terminal of amino acids 578-640 of SEQ ID NO:1 and with at least 70% sequence identity to such a sequence of consecutive amino acids. In some embodiments an amino acid sequence identity referred to herein of at least 70% of any one sequence identified by SEQ ID NO:1-57 or a fragment thereof, refers to a sequence with at least 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 8, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity to this sequence.

"Mammal" includes humans and both domestic animals such as laboratory animals and household pets (*e.g.*, cats, dogs, swine, cattle, sheep, goats, horses, rabbits), and non-domestic animals such as wildlife and the like.

The term "microorganism" as used herein refers to bacteria, fungi, archaea, protists (such as green algae and plankton), planarians and amoebae. Included within this definition are pathogenic microorganisms.

A "native sequence" polypeptide is one which has the same amino acid sequence as a polypeptide derived from nature. Such native sequence polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. Thus, a native sequence polypeptide can have the amino acid sequence of naturally-occurring human polypeptide, murine polypeptide, or polypeptide from any other mammalian species.

"Optional" or "optionally" means that the subsequently described event of circumstances may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, "optionally substituted aryl" means that the aryl substituent may or may not be substituted and that the description includes both substituted aryl substituents and aryl substituents having no substitution.

A "pharmaceutical composition" refers to a formulation of a compound of the disclosure and a medium generally accepted in the art for the delivery of the biologically active compound to mammals, *e.g.*, humans. Such a medium includes all pharmaceutically acceptable carriers, diluents or excipients therefor.

"Pharmaceutically acceptable carrier, diluent or excipient" includes without limitation any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative, dye/colorant, flavor enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizer, isotonic agent, solvent, or emulsifier which has been approved by the United States Food and Drug Administration as being acceptable for use in humans or domestic animals.

"Pharmaceutically acceptable salt" includes both acid and base addition salts. "Pharmaceutically acceptable acid addition salt" refers to those salts which retain the biological effectiveness and properties of the free bases, which are not biologically or otherwise undesirable, and which are formed with inorganic acids such as, but are not limited to, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as, but not limited

to, acetic acid, 2,2-dichloroacetic acid, adipic acid, alginic acid, ascorbic acid, aspartic acid, benzenesulfonic acid, benzoic acid, 4-acetamidobenzoic acid, camphoric acid, camphor-10-sulfonic acid, capric acid, caproic acid, caprylic acid, carbonic acid, cinnamic acid, citric acid, cyclamic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid, ethanesulfonic acid, 2-hydroxyethanesulfonic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, glucoheptonic acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, 2-oxo-glutaric acid, glycerophosphoric acid, glycolic acid, hippuric acid, isobutyric acid, lactic acid, lactobionic acid, lauric acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulfonic acid, mucic acid, naphthalene-1,5-disulfonic acid, naphthalene-2-sulfonic acid, 1-hydroxy-2-naphthoic acid, nicotinic acid, oleic acid, orotic acid, oxalic acid, palmitic acid, pamoic acid, propionic acid, pyroglutamic acid, pyruvic acid, salicylic acid, 4-aminosalicylic acid, sebacic acid, stearic acid, succinic acid, tartaric acid, thiocyanic acid, *p*-toluenesulfonic acid, trifluoroacetic acid, undecylenic acid, and the like. "Pharmaceutically acceptable base addition salt" refers to those salts which retain the biological effectiveness and properties of the free acids, which are not biologically or otherwise undesirable. These salts are prepared from addition of an inorganic base or an organic base to the free acid. Salts derived from inorganic bases include, but are not limited to, the sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Preferred inorganic salts are the ammonium, sodium, potassium, calcium, and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as ammonia, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, diethanolamine, ethanolamine, deanol, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, benethamine, benzathine, ethylenediamine, glucosamine, methylglucamine, theobromine, triethanolamine, tromethamine, purines, piperazine, piperidine, *N*-ethylpiperidine, polyamine resins and the like. Particularly preferred organic bases are isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexylamine, choline and caffeine.

"Prodrug" is meant to indicate a compound that may be converted under physiological conditions or by solvolysis to a biologically active compound of the disclosure. Thus, the term "prodrug" refers to a metabolic precursor of a compound of the disclosure that is pharmaceutically acceptable. A prodrug may be inactive when administered to a subject in need thereof, but is converted *in vivo* to an active compound of the disclosure. Prodrugs are typically rapidly transformed *in vivo* to yield the parent compound of the disclosure, for example, by hydrolysis in blood. The prodrug compound often offers advantages of solubility, tissue compatibility or delayed release in a mammalian organism (see, Bundgard, H., Design of Prodrugs (1985), pp. 7-9, 21-24 (Elsevier, Amsterdam)). A discussion of prodrugs is provided in Higuchi, T., *et al.*, A.C.S. Symposium Series,

Vol. 14, and in Bioreversible Carriers in Drug Design, Ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987. Prodrugs of a compound of the disclosure may be prepared by modifying functional groups present in the compound of the disclosure in such a way that the modifications are cleaved, either in routine manipulation or *in vivo*, to the parent compound of the disclosure. Prodrugs include compounds of the disclosure wherein a hydroxy, amino or mercapto group is bonded to any group that, when the prodrug of the compound of the disclosure is administered to a mammalian subject, cleaves to form a free hydroxy, free amino or free mercapto group, respectively. Examples of prodrugs include, but are not limited to, acetate, formate and benzoate derivatives of alcohol or amide derivatives of amine functional groups in the compounds of the disclosure and the like.

The term "protecting group", as used herein, refers to a labile chemical moiety which is known in the art to protect reactive groups including without limitation, hydroxyl and amino groups, against undesired reactions during synthetic procedures. Hydroxyl and amino groups which protected with a protecting group are referred to herein as "protected hydroxyl groups" and "protected amino groups", respectively. Protecting groups are typically used selectively and/or orthogonally to protect sites during reactions at other reactive sites and can then be removed to leave the unprotected group as is or available for further reactions. Protecting groups as known in the art are described generally in Greene and Wuts, Protective Groups in Organic Synthesis, 3rd edition, John Wiley & Sons, New York (1999). Groups can be selectively incorporated into compounds of the present disclosure as precursors. For example an amino group can be placed into a compound of the disclosure as an azido group that can be chemically converted to the amino group at a desired point in the synthesis. Generally, groups are protected or present as a precursor that will be inert to reactions that modify other areas of the parent molecule for conversion into their final groups at an appropriate time. Further representative protecting or precursor groups are discussed in Agrawal, *et al.*, Protocols for Oligonucleotide Conjugates, Eds, Humana Press; New Jersey, 1994; Vol. 26 pp. 1-72. Examples of "hydroxyl protecting groups" include, but are not limited to, *t*-butyl, t-butoxymethyl, methoxymethyl, tetrahydropyranyl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 2-trimethylsilylethyl, *p*-chlorophenyl, 2,4-dinitrophenyl, benzyl, 2,6-dichlorobenzyl, diphenylmethyl, *p*-nitrobenzyl, triphenylmethyl, trimethylsilyl, triethylsilyl, *t*-butyldimethylsilyl, *t*-butyldiphenylsilyl (TBDPS), triphenylsilyl, benzoylformate, acetate, chloroacetate, trichloroacetate, trifluoroacetate, pivaloate, benzoate, *p*-phenylbenzoate, 9-fluorenylmethyl carbonate, mesylate and tosylate. Examples of "amino protecting groups" include, but are not limited to, carbamate-protecting groups, such as 2-trimethylsilylethoxycarbonyl (Teoc), 1-methyl-1-(4-biphenylyl)ethoxycarbonyl (Bpoc), *t*-butoxycarbonyl (BOC), allyloxycarbonyl (Alloc), 9-fluorenylmethyloxycarbonyl (Fmoc), and benzyloxycarbonyl (Cbz); amide protecting groups, such as formyl, acetyl, trihaloacetyl, benzoyl, and

nitrophenylacetyl; sulfonamide-protecting groups, such as 2-nitrobenzenesulfonyl; and imine and cyclic imide protecting groups, such as phthalimido and dithiasuccinoyl.

Often crystallizations produce a solvate of the compound of the disclosure. As used herein, the term "solvate" refers to an aggregate that comprises one or more molecules of a compound of the disclosure with one or more molecules of solvent. The solvent may be water, in which case the solvate may be a hydrate. Alternatively, the solvent may be an organic solvent. Thus, the compounds of the present disclosure may exist as a hydrate, including a monohydrate, dihydrate, hemihydrate, sesquihydrate, trihydrate, tetrahydrate and the like, as well as the corresponding solvated forms. The compound of the disclosure may be true solvates, while in other cases, the compound of the disclosure may merely retain adventitious water or be a mixture of water plus some adventitious solvent.

"Stable compound" and "stable structure" are meant to indicate a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent.

The term "sequence identity" as known in the art, refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between nucleic acid molecules or between polypeptides, as the case may be, as determined by the number of matches between strings of two or more nucleotide residues or two or more amino acid residues. "Identity" measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (*i.e.*, "algorithms"). The term "similarity" is a related concept, but in contrast to "identity", refers to a sequence relationship that includes both identical matches and conservative substitution matches. Therefore, in cases where there are conservative substitutions, the degree of similarity between two polypeptides will be higher than the percent identity between those two polypeptides.

A "stereoisomer" refers to a compound made up of the same atoms bonded by the same bonds but having different three-dimensional structures, which are not interchangeable. The present disclosure contemplates various stereoisomers and mixtures thereof and includes "enantiomers", which refers to two stereoisomers whose molecules are nonsuperimposeable mirror images of one another.

A "tautomer" refers to a proton shift from one atom of a molecule to another atom of the same molecule. The present disclosure includes tautomers of any said compounds.

"Treating" or "treatment" as used herein covers the treatment of the disease or condition of interest in a mammal, preferably a human, having the disease or condition of interest, and includes:

(i) preventing the disease or condition from occurring in a mammal, in particular, when such mammal is predisposed to the condition but has not yet been diagnosed as having it;

(ii) inhibiting the disease or condition, *i.e.*, arresting its development;

(iii) relieving the disease or condition, *i.e.*, causing regression of the disease or condition; or

(iv) relieving the symptoms resulting from the disease or condition, *i.e.*, relieving pain without addressing the underlying disease or condition.

In one embodiment, the term "VAR2CSA polypeptide" as used herein refers to the extracellular part of a specific Erythrocyte Membrane Protein 1 (PfEMP1) protein expressed by *Plasmodium falciparum* interacting with chondroitin sulfate proteoglycans (CSPG) and characterized by having a sequence of SEQ ID NO:55 or SEQ ID NO:56, or fragments or variants or derivatives thereof with the ability to bind plCSA that could be presented on a proteoglycan (CSPG). In some embodiments, the VAR2CSA polypeptide at least comprises the protein fragment of VAR2CSA, which fragment consist of a sequential amino acid sequence of a) ID1, and b) DBL2Xb. In some embodiments, the VAR2CSA polypeptide at least comprises the protein fragment of VAR2CSA, which fragment consist of a sequential amino acid sequence of a) ID1, and b) DBL2Xb, and c) ID2a. In some embodiments, the VAR2CSA polypeptide competes for binding to plCSA with a VAR2CSA polypeptide consisting of sequential amino acid sequence of a) ID1, and b) DBL2Xb. In some embodiments, the VAR2CSA polypeptide competes for binding to plCSA with a VAR2CSA polypeptide consisting of sequential amino acid sequence of a) ID1, b) DBL2Xb and c) ID2a. In some embodiments, the VAR2CSA polypeptide competes for binding to plCSA with a VAR2CSA polypeptide comprising the amino acid sequence in SEQ ID NO:55 or SEQ ID NO:56. Included within the definition of a VAR2CSA polypeptide are polypeptides described in Salanti A. *et al.* Mol. Micro 2003 Jul;49(1):179-91; in Khunrae P. *et al.*, J Mol Biol. 2010 Apr 2;397(3):826-34, in Srivastava A. *et al.*, Proc Natl Acad Sci U S A. 2010 Mar 16;107(11):4884-9, in Dahlbäck M. et al, J Biol Chem. 2011 May 6;286(18):15908-17, and in Srivastava A. *et al.*, PLoS One. 2011;6(5):e20270.

The terms "variant" or "variants", as used herein, refer to a VAR2CSA polypeptide having an amino acid sequence of SEQ ID NO:55 or SEQ ID NO:56 or fragments of a VAR2CSA polypeptide comprising an amino acid sequence of SEQ ID NO:1-54, which fragments or variants retain the ability to bind plCSA on proteoglycans (CSPG), wherein one or more amino acids have been substituted by another amino acid and/or wherein one or more amino acids have been deleted and/or wherein one or more amino acids have been inserted in the polypeptide and/or wherein one or more amino acids have been added to the polypeptide. Such addition can take place either at the N-terminal end or at the C-terminal end or both. The "variant" or "variants" within this definition still have functional activity in terms of being able to bind plCSA. Accordingly, such polypeptides are VAR2CSA polypeptides, as used herein. In some embodiment a variant has at least 70%, such as at least 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 8, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96,

97, 98, or 99% sequence identity with the sequence of SEQ ID NO:1-57, such as the sequence of SEQ ID NO:1, 3-5, 10, 11, 29, 34, 36-38, 41, 43-45, 48, 53-56, 60-70, 72-75.

The term "vector", as used herein, means any nucleic acid entity capable of the amplification in a host cell. Thus, the vector may be an autonomously replicating vector, *i.e.* a vector, which exists as an extra-chromosomal entity, the replication of which is independent of chromosomal replication, *e.g.* a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated. The choice of vector will often depend on the host cell into which it is to be introduced. Vectors include, but are not limited to plasmid vectors, phage vectors, viruses or cosmid vectors. Vectors usually contain a replication origin and at least one selectable gene, *i.e.*, a gene which encodes a product which is readily detectable or the presence of which is essential for cell growth.

COMPOUNDS

Provided are protein-drug conjugates which are compounds of Formula I:

T–L–P

I

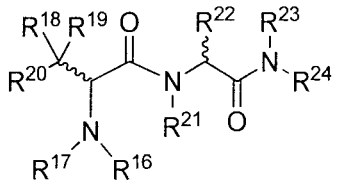
wherein:

T is a targeting moiety comprising a VAR2CSA polypeptide;

L–P is selected from: L^1–P^1 or L^2–P^2;

L^1 is a linker, or L^1 is absent;

P^1 is a monovalent radical of a compound of Formula XIV



XIV

wherein:

R^{16} and R^{17} are independently selected from the group consisting of: H and a saturated or unsaturated moiety having a linear, branched, or non-aromatic cyclic skeleton containing one to ten carbon atoms, and the carbon atoms are optionally substituted with: –OH, –I, –Br, –Cl, –F, –CN, $–CO_2H$, –CHO, –COSH, or $–NO_2$; or R^{17} and R^{20} are fused and form a ring;

R^{18} and R^{19} are independently selected from the group consisting of: H, R^{25}, and ArR^{25}–, or R^{18} and R^{19} are joined to form a ring;

R^{20} is selected from the group consisting of: H, R^{25}, ArR^{25}–, and Ar; or R^{20} and R^{17} are fused and form a ring;

R^{21} is selected from the group consisting of: H, R^{25}, and ArR^{25}–;

R^{22} and R^{23} are independently selected from the group consisting of: H, R^{25}, and ArR^{25}–;

R^{24} is: –Y–(CO)$NHSO_2$–R^{26}

R^{25} is defined as a saturated or unsaturated moiety having a linear, branched, or non-aromatic cyclic skeleton containing one to ten carbon atoms, zero to four nitrogen atoms, zero to four oxygen atoms, and zero to four sulfur atoms, and the carbon atoms are optionally substituted with: =O, =S, OH, $–OR^{28}$, $–O_2CR^{28}$, –SH, $–SR^{28}$, $–SOCR^{28}$, $–NH_2$, $–NHR^{28}$, $–N(R^{28})_2$, $–NHCOR^{28}$, $–NR^{28}COR^{28}$, –I, –Br, –Cl, –F, –CN, $–CO_2H$, $–CO_2R^{28}$, –CHO, $–COR^{28}$, $–CONH_2$, $–CONHR^{28}$, $–CON(R^{28})_2$, –COSH, $–COSR^{28}$, $-NO_2$, $-SO_3H$, $-SOR^{28}$, $-SO_2R^{28}$, wherein R^{28} is a linear, branched or cyclic, one to ten carbon saturated or unsaturated alkyl group;

the ring formed by joining R^{18} and R^{19} is a three to seven member non-aromatic cyclic skeleton within the definition of R^{25},

Y is defined as a moiety selected from the group consisting of: a linear, saturated or unsaturated, one to six carbon alkyl group, optionally substituted with R^{25}, ArR^{25}–, or X; and,

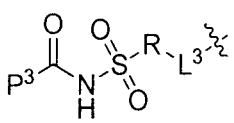
X is defined as a moiety selected from the group consisting of: –OH, $–OR^{25}$, =O, =S, $–O_2CR^{25}$, –SH, $–SR^{25}$, $–SOCR^{25}$, $–NH_2$, $–NHR^{25}$, $–N(R^{25})_2$, $–NHCOR^{25}$, $–NRCOR^{25}$, –I, –Br, –Cl, –F, –CN, $–CO_2H$, $–CO_2R^{25}$, –CHO, $–COR^{25}$, $–CONH_2$, $–CONHR^{25}$, $–CON(R^{25})_2$, –COSH, $–COSR^{25}$, $–NO_2$, $–SO_3H$, $–SOR^{25}$, and $–SO_2R^{25}$;

R^{26} is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, $–COR^{27}$, $–CSR^{27}$, $–OR^{27}$, and $–NHR^{27}$, wherein each R^{27} is, independently, optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, or optionally substituted heteroaryl; and

L^2 is a linker, or L^2 is absent;

P^2 is a biologically active compound; and

L^2–P^2 has the following structure (III):



III

wherein:

R is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, $-COR^{27}$, $-CSR^{27}$, $-OR^{27}$, and $-NHR^{27}$, wherein each R^{27} is, independently, optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, or R is absent;

P^3 is the remaining portion of compound P^2; and

L^3 is optionally the remaining portion of linker L^2 when L^2 is present.

In a preferred embodiment, R is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl, or R is absent.

In some embodiments, L^2 is present and T and L^2 are linked via a peptide bond. In some embodiments, R is present and L^2 is present and L^2 and P^2 are linked via a peptide bond. In some embodiments, L^2 is absent, R is present, and T and P^2 are linked via a peptide bond.

In certain embodiments, one payload molecule is linked to one linker molecule. In certain embodiments, a plurality of payload molecules are linked to the same linker molecule. In certain embodiments, one linker molecule is linked to one targeting moiety. In certain embodiments, a plurality of linker molecules are linked to the same targeting moiety. "Drug-antibody ratio" or "DAR" is meant to indicate the number of drug moieties conjugated to a targeting moiety (an antibody).

TARGETING MOIETY (T)

A targeting moiety can form a bond to a linker unit (L) or a payload compound (P). A targeting moiety can form a bond to the linker moiety or the payload compound via a heteroatom of the targeting moiety. Heteroatoms that may be present on a targeting moiety include sulfur (in one embodiment, from a sulfhydryl group of T), oxygen (in one embodiment, from a carbonyl, carboxyl or hydroxyl group of T) and nitrogen (in one embodiment, from a primary or secondary amino group of T). These heteroatoms can be present on the targeting moiety in the targeting moiety's natural state, or can be introduced into the targeting moiety, for example by chemical modification or recombinant means.

In some embodiments, the targeting moiety has a sulfhydryl group and bonds to the linker moiety via the sulfhydryl group's sulfur atom. In another embodiment, the targeting moiety has one or more lysine residues that can be chemically modified to introduce one or more sulfhydryl groups. The targeting moiety bonds to the linker moiety via the sulfhydryl group. Reagents that can be used to modify lysines include, but are not limited to, *N*-succinimidyl *S*-acetylthioacetate (SATA) and 2-iminothiolane hydrochloride (Traut's Reagent).

In another embodiment, the linker moiety can have one or more carbohydrate groups that can be chemically modified to have one or more sulfhydryl groups. The targeting moiety bonds to the linker moiety via the sulfhydryl group's sulfur atom. In yet another embodiment, the targeting moiety can have one or more carbohydrate groups that can be oxidized to provide an aldehyde (–CHO) group (see, *e.g.*, Laguzza *et al.*, 1989, J. Med. Chem. 32(3):548-55). The corresponding aldehyde can form a bond with a reactive site on a portion of a linker moiety. Reactive sites that can react with a carbonyl group on a targeting moiety include, but are not limited to, hydrazine and hydroxylamine. Other protocols for the modification of proteins for the attachment or association of payload compounds are described in Coligan *et al.*, Current Protocols in Protein Science, vol. 2, John Wiley & Sons (2002).

Provided are compounds of Formula I:

T–L–P

I

wherein T is a targeting moiety comprising a VAR2CSA polypeptide.

The targeting moiety described herein includes within its scope any molecule comprising a VAR2CSA polypeptide as defined herein. In a preferred embodiment, the targeting moiety is a protein comprising a VAR2CSA polypeptide. In another preferred embodiment, the targeting moiety consists essentially of a VAR2CSA polypeptide. In another preferred embodiment, the targeting moiety consists of a VAR2CSA polypeptide. In a preferred embodiment, the VAR2CSA polypeptide is a VAR2CSA polypeptide disclosed in WO2013/117705.

The VAR2CSA protein may be recombinantly produced from any number of host cell types, as will be recognized by one of reasonable skill in the art. In one embodiment, VAR2CSA polypeptide is produced recombinantly using a mammalian cell system. In another embodiment, VAR2CSA polypeptide is produced using a non-mammalian cell system. In one embodiment, VAR2CSA polypeptide is produced using an insect cell system. As will be appreciated by the reasonably skilled artisan, the glycosylation pattern of recombinant VAR2CSA polypeptide produced by different cell types may vary.

In some embodiments, the VAR2CSA polypeptide is not a minimal binding fragment. In some embodiments, the VAR2CSA polypeptide is a minimal binding fragment. In some embodiments, the VAR2CSA polypeptide consists of a sequential amino acid sequence of a) ID1, and b) DBL2Xb, and optionally c) ID2a. In some embodiments the VAR2CSA polypeptide comprises ID2a.

VAR2CSA, part of a malaria protein, can bind to a cancer-specific antigen and extra-cellular CSPG with very high specificity and very high binding strength. VAR2CSA mediates adhesion of parasite-infected cells exclusively to CSA attached to proteoglycans (CSPG) in the placenta of pregnant women. Recombinant protein has been shown to bind with unprecedented high affinity and specificity to plCSA. This may be due to an interaction with plCSA that is not only dependent on the

charged sulfates but also on the CS backbone. CS present in the placenta is believed to be very similar to the CS presented on various cancer cells including cancer stem cells (Salanti *et al.*, WO2013/117705). This is substantiated by the fact that VAR2CSA-expressing parasite infected cells adhere specifically to plCSA on C32 melanoma cells and to human brain cancer cells. By coupling VAR2CSA to an apoptotic or cytotoxic payload the compounds described herein can be used to specifically target and eliminate cancer cells and cancer stem cells. plCSA can be present on a number of protein backbones, *e.g.* CSPG4, CD44, biglycan, decorin, versican, aggrecan (the major CSPG in cartilage), perlecan, syndecan, and others listed in Table 1.

Table 1. Potential molecules targeted by a VAR2CSA polypeptide

Protein ID 1	Protein ID 2	Gene name
NG2	CSPG4	cspg4
Neuroglycan and Neuroglycan-C	CSPG5	ngc 7
Neuropilin-1 CS	NRP-1-CS	NRP1
APLP2 and APP (and when plCSA is added the proteins are called Appicans)	amyloid precursor-like protein 2	APLP2
Snorc		Snorc
Tomoregulin-1	TENB1	TMEFF1
Tomoregulin-2	TENB2	TMEFF2
Thrombomodulin	CD141	THBD
Betaglycan	Transforming growth factor beta receptor III	TGFBR3
Syndecan 1	CD138	SDC1
Syndecan 2	CD362	SDC2
Syndecan 3		SDC3
Syndecan 4	Amphiglycan	SDC4
CSPG8	CSPG8	Cd44
Glypican1-6 (kun 1 og 5)		GPC1-6
Brevican	CSPG7	bcan
lubricin	Proteoglycan 4	PRG4
Dentin matrix protein 1		DMP1
Neurocan	CSPG3	ncan
Versican	CSPG2	vcan
Aggrecan	CSPG1	acan

Protein ID 1	Protein ID 2	Gene name
Bamecan	CSPG6	smc3
SRPX2	Sushi repeat-containing protein	SRPX2
Serglycin	Hematopoietic proteoglycan core protein	SRGN
Decorin	Small leucine-rich proteoglycan (SLRP) family	dcn
Biglycan	Small leucine-rich proteoglycan (SLRP) family	bgn
Lumican	Small leucine-rich proteoglycan (SLRP) family	lum
Fibromodulin	Small leucine-rich proteoglycan (SLRP) family	fmod
Keratocan	Small leucine-rich proteoglycan (SLRP) family	kera
Mimecan	osteoglycin	ogn
Testican 1-3	BM-40/SPARC/ osteonectin family of extracellular calcium-binding proteins	SPOCK1
phosphacan	Receptor-type tyrosine-protein phosphatase zeta	PTPRZ1
Leprecan	Leucine Proline-Enriched Proteoglycan 1	LEPRE1
Perlecan	basement membrane-specific heparan sulfate proteoglycan core protein	HSPG2

VAR2CSA binds plCSA in the intervillous spaces of the placenta with an affinity below 10 nM. Smaller recombinant parts of VAR2CSA have been produced that bind plCSA with characteristics similar to that of the full-length and native VAR2CSA protein. Table 2 lists the plCSA affinity of certain VAR2CSA polypeptides using biosensor technology. Affinity is given as a K_D (nM) value determined in kinetics experiments using a quartz crystal microbalance biosensor (Attana A100). N/A: proteins for which no KD value could be determined, due to a lack of binding to plCSA.

Table 2. plCSA Binding Affinity of Certain VAR2CSA Polypeptides.

	FCR3		3D7
VAR2CSA Fragment	Baculo	*E. coli*	Baculo
FV2	5.2*		8.2
ID1-DBL4ε	8.6*		9.4
ID1-DBL3ε	0.3*		8.5
DBL2X-DBL4ε	2.4*		1.2
DBL1-ID2b	1.5*		
DBL1-ID2a	8.0	3.5	29.5
ID1-ID2a	7.6	18.3	5.7
DBL1X-DBL2Xb		14.6	
DBL1X-DBL2Xa	N/A		
ID1-DBL2Xb			21.8
ID1-DBL2Xa			N/A

* Proteins published in (Dahlbäck *et al.*, JBC, 2011)

Recombinant VAR2CSA protein does not bind other CS such as chondroitin sulfate C (C6S) or highly sulfated GAGs such as heparan sulfate (HS). Recombinant proteins can be produced to bind with high affinity to plCSA in various expression systems, *e.g.*, S2 cells, *T. ni* cells, CHO cells, and *E. coli* strains including BL21 and SHuffle.

A number of VAR2CSA polypeptides smaller than full length VAR2CSA and which bind plCSA with very high affinity (nM) and high specificity have been identified (Salanti *et al.*, WO2013/117705). As shown herein, such a representative VAR2CSA polypeptide (75 kDa) binds strongly at low concentrations to a wide range of cancer cell lines including cutaneous melanoma (C32, MeWo), lung carcinoma (A549), breast carcinoma (HCC1395), osterosarcoma (U205, MNNG/HOS), rhabdomyosarcoma (RH30) and cutaneous T-cell lymphoma (Tables 3 and 4). As a control molecule another VAR2CSA protein was used, which is identical to the minimal binding VAR2CSA construct except for a 151 amino acids truncation in the C-terminal part of the molecule. This truncation removes the plCSA binding. Recombinant VAR2CSA binds all CSPG4 expressing cell lines and cancer cell lines expressing other CSPG molecules having plCSA chains (*e.g.* T-cell lymphoma). Recombinant VAR2CSA protein fails to interact with human red blood cells and peripheral blood mononuclear cells (PBMC) (Table 3).

Table 3. Staining of Cancer Cell Lines and Negative Control Cells Using the Minimal Binding Domain of VAR2CSA (ID1-ID2a). Shown are the mean FITC 30 fluorescence values recorded from a minimum of 5000 cells using a FC500 flowcytometer (Becton Dickinson).

Cell type	Blank	ID1-DBL2Xa	ID1-ID2a
C32	5.77	6.94	63.81
MyLa 2059	5.61	5.61	145.35
MyLa 1850	5.87	5.6	137.86
Cho WT	3.09	4.35	34.79
Cho 745	4.24	4.29	4.38
PBMC	1.34	1.36	1.67
Erythrocytes	1.11	1.17	1.07

Table 4. Staining of cancer cell lines using recombinant VAR2CSA. Shown are the medium score of FITC fluorescence intensity recorded from a minimum of 4 high power field images using a HAL100 Zeiss microscope. NS: No staining; +: weak; ++: medium; +++: strong; ++++: Very strong.

Cell type	Blank	DBL1-ID2a
U2OS	NS	+++
MG63	NS	++++
MDA-MB-231	NS	+++
TC32	NS	+
TC71	NS	++
MNNG	NS	+++
CHLA9	NS	++
CHLA10	NS	++
RH30	NS	+++
RH18	NS	++
PC3	NS	+++

Cells infected with malaria parasites adhere to C32 melanoma cells, probably through a specific interaction between CSPG4 and VAR2CSA. Thus, it is envisioned that the compounds described herein may be used as therapeutic compounds targeting plCSA on various cancer cells. The advantages of targeting plCSA on cancer cells with VAR2CSA polypeptides over other current therapies in development are numerous: 1) The interaction between VAR2CSA and plCSA is of unprecedented high affinity and highly specific; 2) VAR2CSA is an evolutionary refined malaria protein and it is thus unlikely that therapy will break tolerance and cause autoimmune reactions in the patient; 3) VAR2CSA is a stable protein that is well characterized and can be highly expressed in organisms compatible with large-scale protein production; 4) VAR2CSA is a polymorphic protein with a number of serovariants. Repeated therapy could be offered by different serovariants to avoid

issues with neutralizing antibodies; 5) VAR2CSA is naturally exposed extracellularly on the *P. falciparum*-infected the red blood cell and is thus by nature a stable protein in human serum and has been shown to be highly protease resistant.

The compounds described herein utilize the interaction between VAR2CSA polypeptides and plCSA. This interaction is a high affinity interaction and one use of such compounds is to target plCSA expressing cancer cells and cancer stem cells. Accordingly, the compounds described herein may be used to target cancer cells with minimal adverse toxicity to plCSA-negative tissue.

plCSA is also involved in other diseases and pathological conditions like for example arthritis, arthrosis, multiple sclerosis and healing after neural damage, cartilage repair, wound healing, and in psoriasis. Accordingly, the compounds described herein are useful in the treatment of any such disease or condition. For example, the compounds described herein are useful for targeting drugs that block protease mediated degradation of aggrecan during arthritis and arthrosis. The compounds described herein may also be used to target anti-inflammatory drugs to the affected tissues and to deliver inhibitors such as ADAMTS4 and -5 inhibitors. The compounds described herein may be used to target drugs that stimulate the production of aggrecan by chondrocytes.

The compounds described herein may be used to target drugs that degrade CSPG or inhibit CSPG production in affected neural tissue, such as chondroitinase ABC, which cut the sugar chains of the protein core of CSPG molecules; xylocides, which reduce CSPG production; and drugs that inhibit enzymes important for CSPG production such as chondroitin synthase or chondroitin polymerizing factor. Examples of such drugs include: 4-fluoro-glucosamine, *p*-nitrophenyl-beta-D-xyloxide, and 4-methyl-umbelliferyl-beta-D-xylopyranoside.

The compounds described herein may also be used to target and maintain cytokines such as IL1-alpha, which stimulate production of ADAMTS4, which subsequently cleave CSPG.

In some embodiments, the VAR2CSA polypeptide described herein consists of a sequential amino acid sequence of a) ID1, and b) DBL2Xb, and optionally c) ID2a.

In some embodiments, the VAR2CSA polypeptide described herein comprises ID2a.

In some embodiments, the VAR2CSA polypeptide described herein does not comprise ID2a.

In some embodiments, the VAR2CSA polypeptide described herein further comprises an amino acid sequence in the N- or C-terminal, or within the sequence of the protein fragment of VAR2CSA of not more than 100 amino acids, such as not more than 90 amino acids, such as not more than 80 amino acids, such as not more than 70 amino acids, such as not more than 60 amino acids, such as not more than 50 amino acids, such as not more than 40 amino acids, such as not more than 30 amino acids, such as not more than 20 amino acids, such as not more than 18 amino acids, such as not more than 16 amino acids, such as not more than 14 amino acids, such as not more than 12 amino acids, such as not more than 10 amino acids, such as not more than 8 amino acids, such as not more than 6 amino acids, such as not more than 4 amino acids, such as not more than 2 amino acids derived

from any part of a VAR2CSA polypeptide as defined herein, which is not part of ID1, DBL2Xb, or ID2a.

In some embodiments, the VAR2CSA polypeptide described herein further comprises an amino acid sequence in the N- or C-terminal, or within the sequence of the protein fragment of VAR2CSA of not more than 100 amino acids, such as not more than 90 amino acids, such as not more than 80 amino acids, such as not more than 70 amino acids, such as not more than 60 amino acids, such as not more than 50 amino acids, such as not more than 40 amino acids, such as not more than 30 amino acids, such as not more than 20 amino acids, such as not more than 18 amino acids, such as not more than 16 amino acids, such as not more than 14 amino acids, such as not more than 12 amino acids, such as not more than 10 amino acids, such as not more than 8 amino acids, such as not more than 6 amino acids, such as not more than 4 amino acids, such as not more than 2 amino acids, which amino acid sequence is not derived from any part of a VAR2CSA polypeptide as defined herein.

In some embodiments, the VAR2CSA polypeptide described herein binds chondroitin sulfate A (CSA) on proteoglycans (CSPG) with an affinity as measured by a K_D lower than 100 nM, such as lower than 80 nM, such as lower than 70 nM, such as lower than 60 nM, such as lower than 50 nM, such as lower than 40 nM, such as lower than 30 nM, such as lower than 26 nM, such as lower than 24 nM, such as lower than 22 nM, such as lower than 20 nM, such as lower than 18 nM, such as lower than 16 nM, such as lower than 14 nM, such as lower than 12 nM, such as lower than 10 nM, such as lower than 9 nM, such as lower than 8 nM, such as lower than 7 nM, such as lower than 6 nM, or lower than 4nM.

In some embodiments, the VAR2CSA polypeptide described herein binds plCSA on proteoglycans (CSPG) with an affinity as measured by a K_D lower than 100 nM, such as lower than 80 nM, such as lower than 70 nM, such as lower than 60 nM, such as lower than 50 nM, such as lower than 40 nM, such as lower than 30 nM, such as lower than 26 nM, such as lower than 24 nM, such as lower than 22 nM, such as lower than 20 nM, such as lower than 18 nM, such as lower than 16 nM, such as lower than 14 nM, such as lower than 12 nM, such as lower than 10 nM, such as lower than 9 nM, such as lower than 8 nM, such as lower than 7 nM, such as lower than 6 nM, or lower than 4nM.

In some embodiments the VAR2CSA polypeptide described herein comprises an amino acid sequence having at least 70% sequence identity with any one amino acid sequence of 1-577 of SEQ ID NO:1, 1-592 of SEQ ID NO:3, 1-579 of SEQ ID NO:4, 1-576 of SEQ ID NO:5, 1-586 of SEQ ID NO:10, 1-579 of SEQ ID NO:11, 1-565 of SEQ ID NO:29, 1-584 of SEQ ID NO:34, 1-569 of SEQ ID NO:36, 1-575 of SEQ ID NO:37, 1-592 of SEQ ID NO:38, 1-603 of SEQ ID NO:41, 1-588 of SEQ ID NO:43, 1-565 of SEQ ID NO:44, 1-589 of SEQ ID NO:45, 1-573 of SEQ ID NO:48, 1-583 of SEQ ID NO:53, or 1-569 of SEQ ID NO:54.

In some embodiments the VAR2CSA polypeptide described herein comprises an amino acid sequence having at least 70% sequence identity with an amino acid sequence of 578-640 of SEQ ID

NO:1, 593-656 of SEQ ID NO:3, 580-643 of SEQ ID NO:4, 577-640 of SEQ ID NO:5, 587-650 of SEQ ID NO:10, 580-643 of SEQ ID NO:11, 566-628 of SEQ ID NO:29, 585-647 of SEQ ID NO:34, 570-632 of SEQ ID NO:36, 576-639 of SEQ ID NO:37, 593-655 of SEQ ID NO:38, 604-667 of SEQ ID NO:41, 589-652 of SEQ ID NO:43, 566-628 of SEQ ID NO:44, 590-653 of SEQ ID NO:45, 574-637 of SEQ ID NO:48, 584-646 of SEQ ID NO:53, or 570-632 of SEQ ID NO:54.

In some embodiments the VAR2CSA polypeptide described herein comprises an amino acid sequence having at least 70% sequence identity with an amino acid sequence of SEQ ID NO:2, 6, 8, 9, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 30, 31, 32, 33, 35, 39, 40, 42, 46, 47, 49, 50, 51, or 52.

In some embodiments the VAR2CSA polypeptide described herein consists of an 30 amino acid sequence having at least 70% sequence identity with any one amino acid sequence of 1-577 of SEQ ID NO:1, 1-592 of SEQ ID NO:3, 1-579 of SEQ ID NO:4, 1-576 of SEQ ID NO:5, 1-586 of SEQ ID NO:10, 1-579 of SEQ ID NO:11, 1-565 of SEQ ID NO:29, 1-584 of SEQ ID NO:34, 1-569 of SEQ ID NO:36, 1-575 of SEQ ID NO:37, 1-592 of SEQ ID NO:38, 1-603 of SEQ ID NO:41, 1-588 of SEQ ID NO:43, 1-565 of SEQ ID NO:44, 1-589 of SEQ ID NO:45, 1-573 of SEQ ID NO:48, 1-583 of SEQ ID NO:53, or 1-569 of SEQ ID NO:54.

In some embodiments the VAR2CSA polypeptide described herein consists of an amino acid sequence selected from the list consisting of SEQ ID NO:1, 3-5, 10, 11, 29, 34, 36-38, 41, 43-45, 48, 53, and 54.

In some embodiments the VAR2CSA polypeptide described herein consists of an amino acid sequence having a length of less than 700 amino acids, such as less than 690 amino acids, such as less than 680 amino acids, such as less than 670 amino acids, such as less than 660 amino acids, such as less than 650 amino acids, such as less than 640 amino acids, such as less than 630 amino acids, such as less than 620 amino acids, such as less than 610 amino acids, such as less than 600 amino acids, such as less than 590 amino acids, such as less than 580 amino acids, such as less than 570 amino acids.

In some embodiments the VAR2CSA polypeptide described herein has a molecular mass of less than about 100 kDa under non-reducing conditions on an SDS-PAGE.

In some embodiments the VAR2CSA polypeptide described herein is a recombinant protein.

In some embodiments the VAR2CSA polypeptide described herein is non-glycosylated.

In some embodiments the VAR2CSA polypeptide described herein is glycosylated.

In some aspects of the present invention, the VAR2CSA polypeptide described herein comprises a sequence as defined by one or more sequences selected from SEQ ID NO 57 or a functional variant or fragment thereof.

In some embodiments the VAR2CSA polypeptide described herein comprises a protease inhibitor, such as basic pancreatic trypsin inhibitor (BPTI) in the terminal, such as the *N*-terminal of the protein sequence, such as a sequence defined by SEQ ID NO:57.

VAR2CSA Polypeptide Modifications

Conservative modifications to the amino acid sequence of SEQ ID NO: 1-56 (and the corresponding modifications to the encoding nucleotides) will produce VAR2CSA polypeptides having functional and chemical characteristics similar to those of naturally occurring VAR2CSA polypeptides. In contrast, substantial modifications in the functional and/or chemical characteristics of a VAR2CSA polypeptide may be accomplished by selecting substitutions in the amino acid sequence of SEQ ID NO: 1-56 that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with a nonnative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Furthermore, any native residue in the polypeptide may also be substituted with alanine, as has been previously described for "alanine scanning mutagenesis" (see, for example, MacLennan *et al.*, 1998, Acta Physiol. Scand. Suppl. 643:55-67; Sasaki *et al.*, 1998, Adv. Biophys. 35:1-24, which discuss alanine scanning mutagenesis). Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. For example, amino acid substitutions can be used to identify important residues of a VAR2CSA polypeptide, or to increase or decrease the affinity of a VAR2CSA polypeptide described herein.

Naturally occurring residues may be divided into classes based on common side chain properties: 1) hydrophobic: norleucine, Met, Ala, Val, Leu, Ile; 2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; 3) acidic: Asp, Glu; 4) basic: His, Lys, Arg; 5) residues that influence chain orientation: Gly, Pro; and 6) aromatic: Trp, Tyr, Phe. Non-conservative substitutions may involve, for example, the exchange of a member of one of these classes for a member from another class. Such substituted residues may be introduced into regions of the *Plasmodium falciparum* VAR2CSA polypeptide that are homologous with non-*Plasmodium falciparum* VAR2CSA polypeptides, or into the non-homologous regions of the molecule. In making such changes, the hydropathic index of amino acids may be considered.

Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics, these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-

3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5). The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is understood in the art. Kyte *et al.*, J. Mol. Biol., 157:105-131 (1982). It is known that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, the substitution of amino acids whose hydropathic indexes are within ±2 is preferred, those that are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity, particularly where the biologically functionally equivalent protein or peptide thereby created is intended for use in immunological embodiments. The greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, *i.e.*, with a biological property of the protein. The following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0±1); glutamate (+3.0±1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (−0.4); proline (−0.5±1); alanine (−0.5); histidine (−0.5); cysteine (−1.0); methionine (−1.3); valine (−1.5); leucine (−1.8); isoleucine (−1.8); tyrosine (−2.3); phenylalanine (−2.5); tryptophan (−3.4). In making changes based upon similar hydrophilicity values, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those that are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred. One may also identify epitopes from primary amino acid sequences on the basis of hydrophilicity. These regions are also referred to as "epitopic core regions."

A skilled artisan will be able to determine suitable variants of the polypeptide as set forth in SEQ ID NO:1-57 using well known techniques. For identifying suitable areas of the molecule that may be changed without destroying activity, one skilled in the art may target areas not believed to be important for activity. For example, when similar polypeptides with similar activities from the same species or from other species are known, one skilled in the art may compare the amino acid sequence of a VAR2CSA polypeptide to such similar polypeptides. With such a comparison, one can identify residues and portions of the molecules that are conserved among similar polypeptides. It will be appreciated that changes in areas of a VAR2CSA polypeptide that are not conserved relative to such similar polypeptides would be less likely to adversely affect the biological activity and/or structure of the VAR2CSA polypeptide. One skilled in the art would also know that, even in relatively conserved regions, one may substitute chemically similar amino acids for the naturally occurring residues while retaining activity (conservative amino acid residue substitutions). Therefore, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in a VAR2CSA polypeptide that correspond to amino acid residues that are important for activity or structure in similar polypeptides. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues of VAR2CSA polypeptides described herein. One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view of that information, one skilled in the art may predict the alignment of amino acid residues of a VAR2CSA polypeptide with respect to its three dimensional structure.

One skilled in the art may choose not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. The variants can then be screened using activity assays as described herein. Such variants could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change would be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

A number of scientific publications have been devoted to the prediction of secondary structure. See Moult J., Curr. Op. in Biotech., 7(4):422-427 (1996), Chou *et al.*, Biochemistry, 13(2):222-245 (1974); Chou *et al.*, Biochemistry, 113(2):211-222 (1974); Chou *et al.*, Adv. Enzymol. Relat. Areas Mol. Biol, 47:45-148 (1978); Chou *et al.*, Ann. Rev. Biochem., 47:251-276 and Chou *et al.*, Biophys. J., 26:367-384 (1979). Moreover, computer programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or proteins, which have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural data base (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within a polypeptide's or protein's structure. See Holm *et al.*, Nucl. Acid. Res., 27(1):244-247 (1999). It has been suggested (Brenner *et al.*, Curr. Op. Struct. Biol., 7(3):369-376 (1997)) that there are a limited number of folds in a given polypeptide or protein and that once a critical number of structures have been resolved, structural prediction will gain dramatically in accuracy.

Additional methods of predicting secondary structure include "threading" (Jones, D., Curr. Opin. Struct. Biol., 7(3):377-87 (1997); Sippl *et al.*, Structure, 4(1):15-9 (1996)), "profile analysis" (Bowie *et al.*, Science, 253:164-170 (1991); Gribskov *et al.*, Meth. Enzymol., 183:146-159 (1990);

Gribskov *et al.*, Proc. Nat. Acad. Sci., 84(13):4355-4358 (1987)), and "evolutionary linkage" (See Home, *supra*, and Brenner, *supra*).

Identity and similarity of related polypeptides can be readily calculated by known methods. Such methods include, but are not limited to, those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991; and Carillo *et al.*, SIAM J. Applied Math., 48:1073 (1988).

Preferred methods to determine identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are described in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package, including GAP (Devereux *et al.*, Nucl. Acid. Res., 12:387 (1984); Genetics Computer Group, University of Wisconsin, Madison, Wis.), BLASTP, BLASTN, and FASTA (Altschul *et al.*, J. Mol. Biol., 215:403-410 (1990)). The BLASTX program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul *et al.* NCB/NLM/NIH Bethesda, Md. 20894; Altschul *et al.*, *supra*). The well known Smith Waterman algorithm may also be used to determine identity.

Certain alignment schemes for aligning two amino acid sequences may result in the matching of only a short region of the two sequences, and this small aligned region may have very high sequence identity even though there is no significant relationship between the two full length sequences. Accordingly, in a preferred embodiment, the selected alignment method (GAP program) will result in an alignment that spans at least 50 contiguous amino acids of the target polypeptide.

For example, using the computer algorithm GAP (Genetics Computer Group, University of Wisconsin, Madison, WI), two polypeptides for which the percent sequence identity is to be determined are aligned for optimal matching of their respective amino acids (the "matched span", as determined by the algorithm). A gap opening penalty (which is calculated as 3 times the average diagonal; the "average diagonal" is the average of the diagonal of the comparison matrix being used; the "diagonal" is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension penalty (which is usually one tenth of the gap opening penalty), as well as a comparison matrix such as PAM 250 or BLOSUM 62 are used in conjunction with the algorithm. A standard comparison matrix (see Dayhoff *et al.*, Atlas of Protein Sequence and Structure, vol. 5, supp.3 (1978) for the PAM 250 comparison matrix; Henikoff *et al.*, Proc. Natl.

Acad. Sci USA, 89:10915-10919 (1992) for the BLOSUM 62 comparison matrix) is also used by the algorithm.

Preferred parameters for a polypeptide sequence comparison include the following: Algorithm: Needleman *et al.*, J. Mol. Biol, 48:443-453 (1970); Comparison matrix: BLOSUM 62 from Henikoff *et al.*, Proc. Natl. Acad. Sci. USA, 89:10915-10919 (1992); Gap Penalty: 12, Gap Length Penalty: 4, Threshold of Similarity: 0. The GAP program is useful with the above parameters. The aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps) using the GAP algorithm. Preferred parameters for nucleic acid molecule sequence comparisons include the following: Algorithm: Needleman *et al.*, J. Mol Biol., 48:443-453 (1970); Comparison matrix: matches = +10, mismatch = 0, Gap Penalty: 50, Gap Length Penalty: 3. The GAP program is also useful with the above parameters. The aforementioned parameters are the default parameters for nucleic acid molecule comparisons.

Other exemplary algorithms, gap opening penalties, gap extension penalties, comparison matrices, thresholds of similarity, *etc.* may be used, including those set forth in the Program Manual, Wisconsin Package, Version 9, September, 1997. The particular choices to be made will be apparent to those of skill in the art and will depend on the specific comparison to be made, such as DNA to DNA, protein to protein, protein to DNA; and additionally, whether the comparison is between given pairs of sequences (in which case GAP or BestFit are generally preferred) or between one sequence and a large database of sequences (in which case FASTA or BLASTA are preferred).

The amino acid sequence alterations may be accomplished by a variety of techniques. Modification of the nucleic acid sequence may be by site-specific mutagenesis. Techniques for site-specific mutagenesis are well known in the art and are described in, for example, Zoller and Smith (DNA 3:479-488, 1984) or "Splicing by extension overlap", Horton *et al.*, Gene 77, 1989, pp. 61-68. Thus, using the nucleotide and amino acid sequences of VAR2CSA, one may introduce the alteration(s) of choice. Likewise, procedures for preparing a DNA construct using polymerase chain reaction using specific primers are well known to persons skilled in the art (*cf.* PCR Protocols, 1990, Academic Press, San Diego, CA, USA).

The polypeptides described herein can also comprise non-naturally occurring amino acid residues. Non-naturally occurring amino acids include, without limitation, beta-alanine, desaminohistidine, *trans*-3-methylproline, 2,4-methanoproline, *cis*-4-hydroxyproline, *trans*-4-hydroxyproline, *N*-methylglycine, allo-threonine, methylthreonine, hydroxyethylcysteine, hydroxyethylhomocysteine, nitroglutamine, homoglutamine, pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, 3,3-dimethylproline, *tert*-leucine, nor-valine, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenylalanine. Several methods are known in the art for incorporating non-naturally occurring amino acid residues into polypeptides. For example, an *in vitro* system can be employed wherein nonsense mutations are

suppressed using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is carried out in a cell-free system comprising an *E. coli* S30 extract and commercially available enzymes and other reagents. Polypeptides are purified by chromatography. See, for example, Robertson *et al.*, J. Am. Chem. Soc. 113:2722, 1991; Ellman *et al.*, Methods Enzymol. 202:301, 1991; Chung *et al.*, Science 259:806-9, 1993; and Chung *et al.*, Proc. Natl. Acad. Sci. USA 90:10145-9, 1993). In a second method, translation is carried out in Xenopus oocytes by microinjection of mutated mRNA and chemically aminoacylated suppressor tRNAs (Turcatti *et al.*, J. Biol. Chem. 271:19991-8, 1996). Within a third method, *E. coli* cells are cultured in the absence of a natural amino acid that is to be replaced (*e.g.*, phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (*e.g.*, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the polypeptide in place of its natural counterpart. See, Koide *et al.*, Biochem. 33:7470-6, 1994. Naturally occurring amino acid residues can be converted to non-naturally occurring species by *in vitro* chemical modification. Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions (Wynn and Richards, Protein Sci. 2:395-403, 1993).

VAR2CSA Nucleic Acid Constructs

The VAR2CSA polypeptides described herein may be produced by means of recombinant nucleic acid techniques. In general, a cloned wild-type VAR2CSA nucleic acid sequence is modified to encode the desired protein. This modified sequence is then inserted into an expression vector, which is in turn transformed or transfected into host cells. Higher eukaryotic cells, in particular cultured mammalian cells, may be used as host cells. Procaryotic cells such as *Lactococcus lactis* or *E. coli* can also be used to express the polypeptides as long as these prokaryotes are able to produce disulfide bonds or the protein is or may be refolded correctly. In addition, Yeast strains can also be used to express the protein, here among *Saccharomyces cerevisiae* and *P. Pichia.*

The nucleic acid construct encoding the VAR2CSA polypeptides described herein may suitably be of genomic or cDNA origin, for instance obtained by preparing a genomic or cDNA library and screening for DNA sequences coding for all or part of the polypeptide by hybridization using synthetic oligonucleotide probes in accordance with standard techniques (*cf.* Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd. Ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989).

The nucleic acid construct encoding a VAR2CSA polypeptide may also be prepared synthetically by established standard methods, *e.g.* the phosphoamidite method described by Beaucage and Caruthers, Tetrahedron Letters 22 (1981), 1859-1869, or the method described by Matthes *et al.*, EMBO Journal 3 (1984), 801-805. According to the phosphoamidite method,

oligonucleotides are synthesized, *e.g.* in an automatic DNA synthesizer, purified, annealed, ligated and cloned in suitable vectors. The DNA sequences encoding the *Plasmodium falciparum* VAR2CSA polypeptides described herein may also be prepared by polymerase chain reaction using specific primers, for instance as described in US 4,683,202, Saiki *et al.*, Science 239 (1988), 487-491, or Sambrook *et al.*, *supra.*

Furthermore, the nucleic acid construct may be of mixed synthetic and genomic, mixed synthetic and cDNA or mixed genomic and cDNA origin prepared by ligating fragments of synthetic, genomic or cDNA origin (as appropriate), the fragments corresponding to various parts of the entire nucleic acid construct, in accordance with standard techniques.

The nucleic acid construct is preferably a DNA construct. DNA sequences for use in producing VAR2CSA polypeptides described herein will typically encode a pre-pro polypeptide at the amino-terminus of VAR2CSA to obtain proper posttranslational processing and secretion from the host cell.

The DNA sequences encoding the *Plasmodium falciparum* VAR2CSA polypeptides described herein are usually inserted into a recombinant vector which may be any vector, which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, *i.e.* a vector, which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, *e.g.* a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

The vector is preferably an expression vector in which the DNA sequence encoding the *Plasmodium falciparum* VAR2CSA polypeptides described herein is operably linked to additional segments required for transcription of the DNA. In general, the expression vector is derived from plasmid or viral DNA, or may contain elements of both. The term, "operably linked" indicates that the segments are arranged so that they function in concert for their intended purposes, *e.g.* transcription initiates in a promoter and proceeds through the DNA sequence coding for the polypeptide.

Expression vectors for use in expressing VAR2CSA polypeptides described herein will comprise a promoter capable of directing the transcription of a cloned gene or cDNA. The promoter may be any DNA sequence, which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell.

Examples of suitable promoters for directing the transcription of the DNA encoding the *Plasmodium falciparum* VAR2CSA polypeptide in mammalian cells include the SV40 promoter (Subramani *et al.*, Mol. Cell Biol. 1 (1981), 854-864), the MT-1 (metallothionein gene) promoter (Palmiter *et al.*, Science 222 (1983), 809-814), the CMV promoter (Boshart *et al.*, Cell 41:521-530,

1985) and the adenovirus 2 major late promoter (Kaufman and Sharp, Mol. Cell. Biol, 2:1304-1319, 1982).

Examples of a suitable promoter for use in insect cells include the polyhedrin promoter (US 4,745,051; Vasuvedan *et al.*, FEBS Lett. 311, (1992) 7-11), the P10 promoter (J.M. Vlak *et al.*, J. Gen. Virology 69, 1988, pp. 765-776), the *Autographa californica* polyhedrosis virus basic protein promoter (EP 397 485), the baculovirus immediate early gene 1 promoter (US 5,155,037; US 5,162,222), and the baculovirus 39K delayed-early gene promoter (US 5,155,037; US 5,162,222).

Examples of suitable promoters for use in yeast host cells include promoters from yeast glycolytic genes (Hitzeman *et al.*, J. Biol. Chem. 255 (1980), 12073-12080; Alber and Kawasaki, J. Mol. Appl. Gen. 1 (1982), 419-434) and alcohol dehydrogenase genes (Young *et al.*, in Genetic Engineering of Microorganisms for Chemicals (Hollaender et al, eds.), Plenum Press, New York, 1982), and the TPI1 (US 4,599,311) and ADH2-4c (Russell *et al.*, Nature 304 (1983), 652-654) promoters.

Examples of suitable promoters for use in filamentous fungus host cells include, for instance, the ADH3 promoter (McKnight *et al.*, The EMBO J. 4 (1985), 2093-2099) and the tpiA promoter. Examples of other useful promoters are those derived from the gene encoding *A. oryzae* TAKA amylase, *Rhizomucor miehei* aspartic proteinase, *A. niger* neutral alpha-amylase, *A. niger* acid stable alpha-amylase, *A. niger* or A. awamori glucoamylase (gluA), Rhizomucor miehei lipase, *A. oryzae* alkaline protease, *A. oryzae* triose phosphate isomerase or *A. nidulans* acetamidase. Preferred are the TAKA-amylase and gluA promoters. Suitable promoters are mentioned in, *e.g.* EP 238 023 and EP 383 779.

The DNA sequences encoding the *Plasmodium falciparum* VAR2CSA polypeptides described herein may also, if necessary, be operably connected to a suitable terminator, such as the human growth hormone terminator (Palmiter *et al.*, Science 222, 1983, pp. 809-814) or the TPI1 (Alber and Kawasaki, J. Mol. Appl. Gen. 1, 1982, pp. 419-434) or ADH3 (McKnight *et al.*, The EMBO J. 4, 1985, pp. 2093-2099) terminators. Expression vectors may also contain a set of RNA splice sites located downstream from the promoter and upstream from the insertion site for the VAR2CSA sequence itself. Preferred RNA splice sites may be obtained from adenovirus and/or immunoglobulin genes. Also contained in the expression vectors is a polyadenylation signal located downstream of the insertion site. Particularly preferred polyadenylation signals include the early or late polyadenylation signal from SV40 (Kaufman and Sharp, *ibid.*), the polyadenylation signal from the adenovirus Elb region, the human growth hormone gene terminator (DeNoto *et al.* Nucl. Acids Res. 9:3719-3730, 1981) and the polyadenylation signal from *Plasmodium falciparum*, human or bovine genes. The expression vectors may also include a noncoding viral leader sequence, such as the adenovirus 2 tripartite leader, located between the promoter and the RNA splice sites; and enhancer sequences, such as the SV40 enhancer.

To direct the *Plasmodium falciparum* VAR2CSA polypeptides described herein into the secretory pathway of the host cells, a secretory signal sequence (also known as a leader sequence, pre-pro sequence or pre sequence) may be provided in the recombinant vector. The secretory signal sequence is joined to the DNA sequences encoding the *Plasmodium falciparum* VAR2CSA polypeptides described herein in the correct reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the peptide. The secretory signal sequence may be that normally associated with the protein, or may be from a gene encoding another secreted protein.

For secretion from yeast cells, the secretory signal sequence may encode any signal peptide, which ensures efficient direction of the expressed *Plasmodium falciparum* VAR2CSA polypeptides described herein into the secretory pathway of the cell. The signal peptide may be naturally occurring signal peptide, or a functional part thereof, or it may be a synthetic peptide. Suitable signal peptides have been found to be the alpha-factor signal peptide (*cf.* US 4,870,008), the signal peptide of mouse salivary amylase (*cf.* O. Hagenbuchle *et al.*, Nature 289, 1981, pp. 643-646), a modified carboxypeptidase signal peptide (*cf.* L.A. Valls *et al.*, Cell 48, 1987, pp. 887-897), the yeast BAR1 signal peptide (*cf.* WO 87/02670), or the yeast aspartic protease 3 (YAP3) signal peptide (*cf.* M. Egel-Mitani *et al.*, Yeast 6, 1990, pp. 127-137).

For efficient secretion in yeast, a sequence encoding a leader peptide may also be inserted downstream of the signal sequence and upstream of the DNA sequence encoding the *Plasmodium falciparum* VAR2CSA polypeptides described herein. The function of the leader peptide is to allow the expressed peptide to be directed from the endoplasmic reticulum to the Golgi apparatus and further to a secretory vesicle for secretion into the culture medium (*i.e.* exportation of the *Plasmodium falciparum* VAR2CSA polypeptides described herein across the cell wall or at least through the cellular membrane into the periplasmic space of the yeast cell). The leader peptide may be the yeast alpha-factor leader (the use of which is described in *e.g.* US 4,546,082, US 4,870,008, EP 16 201, EP 123 294, EP 123 544 and EP 163 529). Alternatively, the leader peptide may be a synthetic leader peptide, which is to say a leader peptide not found in nature. Synthetic leader peptides may, for instance, be constructed as described in WO 89/02463 or WO 92/11378.

For use in filamentous fungi, the signal peptide may conveniently be derived from a gene encoding an Aspergillus sp. amylase or glucoamylase, a gene encoding a *Rhizomucor miehei* lipase or protease or a *Humicola lanuginosa* lipase. The signal peptide is preferably derived from a gene encoding *A. oryzae* TAKA amylase, *A. niger* neutral alpha-amylase, *A. niger* acid-stable amylase, or *A. niger* glucoamylase. Suitable signal peptides are disclosed in, *e.g.* EP 238 023 and EP 215 594.

For use in insect cells, the signal peptide may conveniently be derived from an insect gene (*cf.* WO 90/05783), such as the lepidopteran *Manduca sexta* adipokinetic hormone precursor signal peptide (*cf.* US 5,023,328).

The procedures used to ligate the DNA sequences coding for the *Plasmodium falciparum* VAR2CSA polypeptides described herein, the promoter and optionally the terminator and/or secretory signal sequence, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to persons skilled in the art (*cf.*, for instance, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, New York, 1989).

Methods of transfecting mammalian cells and expressing DNA sequences introduced in the cells are described in *e.g.* Kaufman and Sharp, J. Mol. Biol. 159 (1982), 601-621; Southern and Berg, J. Mol. Appl. Genet. 1 (1982), 327-341; Loyter *et al.*, Proc. Natl. Acad. Sci. USA 79 (1982), 422-426; Wigler *et al.*, Cell 14 (1978), 725; Corsaro and Pearson, Somatic Cell Genetics 7 (1981), 603, Graham and van der Eb, Virology 52 (1973), 456; and Neumann *et al.*, EMBO J. 1 (1982), 841-845.

Cloned DNA sequences are introduced into cultured mammalian cells by, for example, calcium phosphate-mediated transfection (Wigler *et al.*, Cell 14:725-732, 1978; Corsaro and Pearson, Somatic Cell Genetics 7:603-616, 1981; Graham and Van der Eb, Virology 52d:456-467, 1973) or electroporation (Neumann *et al.*, EMBO J. 1:841-845, 1982). To identify and select cells that express the exogenous DNA, a gene that confers a selectable phenotype (a selectable marker) is generally introduced into cells along with the gene or cDNA of interest. Preferred selectable markers include genes that confer resistance to drugs such as neomycin, hygromycin, and methotrexate. The selectable marker may be an amplifiable selectable marker. A preferred amplifiable selectable marker is a dihydrofolate reductase (DHFR) sequence. Selectable markers are reviewed by Thilly (Mammalian Cell Technology, Butterworth Publishers, Stoneham, MA). The person skilled in the art will easily be able to choose suitable selectable markers.

Selectable markers may be introduced into the cell on a separate plasmid at the same time as the gene of interest, or they may be introduced on the same plasmid. If on the same plasmid, the selectable marker and the gene of interest may be under the control of different promoters or the same promoter, the latter arrangement producing a dicistronic message. Constructs of this type are known in the art (for example, Levinson and Simonsen, U.S. 4,713,339). It may also be advantageous to add additional DNA, known as "carrier DNA," to the mixture that is introduced into the cells.

After the cells have taken up the DNA, they are grown in an appropriate growth medium, typically 1-2 days, to begin expressing the gene of interest. In this context, "appropriate growth medium" means a medium containing nutrients and other components required for the growth of cells and the expression of the *Plasmodium falciparum* VAR2CSA polypeptide of interest. Media generally include a carbon source, a nitrogen source, essential amino acids, essential sugars, vitamins, salts, phospholipids, protein and growth factors. Drug selection is then applied to select for the growth of cells that are expressing the selectable marker in a stable fashion. For cells that have been transfected with an amplifiable selectable marker the drug concentration may be increased to select for an increased copy number of the cloned sequences, thereby in-creasing expression levels. Clones of

stably transfected cells are then screened for expression of the *Plasmodium falciparum* VAR2CSA polypeptide of interest.

The host cell into which the DNA sequences encoding the *Plasmodium falciparum* VAR2CSA polypeptides described herein is introduced may be any cell which is capable of producing the posttranslational modified polypeptides, and includes yeast, fungi and higher eukaryotic cells.

Examples of mammalian cell lines for use in the present invention are the COS-1 (ATCC CRL 1650), baby hamster kidney (BHK) and 293 (ATCC CRL 1573; Graham *et al.*, J. Gen. Virol. 36:59-72, 1977) cell lines. A preferred BHK cell line is the tk-ts13 BHK cell line (Waechter and Baserga, Proc. Natl. Acad. Sci. USA 79:1106-1110, 1982), hereinafter referred to as BHK 570 cells. The BHK 570 cell line has been deposited with the American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852, under ATCC accession number CRL 10314. A tk-ts13 BHK cell line is also available from the ATCC under accession number CRL 1632. In addition, a number of other cell lines may be used within the present invention, including Rat Hep I (rat hepatoma; ATCC CRL 1600), Rat Hep II (rat hepatoma; ATCC CRL 1548), TCMK (ATCC CCL 139), human lung (ATCC HB 8065), NCTC 1469 (ATCC CCL 9.1), CHO (ATCC CCL 61) and DUKX cells (Urlaub and Chasin, Proc. Natl. Acad. Sci. USA 77:4216-4220, 1980).

Examples of suitable yeasts cells include cells of Saccharomyces spp. or Schizosaccharomyces spp., in particular strains of *Saccharomyces cerevisiae* or *Saccharomyces kluyveri*. Methods for transforming yeast cells with heterologous DNA and producing heterologous polypeptides there from are described, *e.g.* in US 4,599,311, US 4,931,373, US 4,870,008, 5,037,743, and US 4,845,075. Transformed cells are selected by a phenotype determined by a selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient, *e.g.* leucine. A preferred vector for use in yeast is the POT1 vector disclosed in US 4,931,373. The DNA sequences encoding the *Plasmodium falciparum* VAR2CSA polypeptides described herein may be preceded by a signal sequence and optionally a leader sequence, *e.g.* as described above. Further examples of suitable yeast cells are strains of Kluyveromyces, such as *K. lactis*, Hansenula, *e.g. H. polymorpha*, or Pichia, *e.g. P. pastoris* (*cf.* Gleeson *et al.*, J. Gen. Microbiol. 132, 1986, pp. 3459-3465; US 4,882,279).

Examples of other fungal cells are cells of filamentous fungi, *e.g.* Aspergillus spp., Neurospora spp., Fusarium spp. or Trichoderma spp., in particular strains of *A. oryzae*, *A. nidulans* and *A. niger*. The use of Aspergillus spp. for the expression of proteins is described in, *e.g.*, EP 272 277, EP 238 023, EP 184 438 The transformation of F. oxysporum may, for instance, be carried out as described by Malardier *et al.*, 1989, Gene 78: 147-156. The transformation of Trichoderma spp. may be performed for instance as described in EP 244 234.

When a filamentous fungus is used as the host cell, it may be transformed with the DNA construct described herein, conveniently by integrating the DNA construct in the host chromosome to obtain a recombinant host cell. This integration is generally considered to be an advantage as the DNA sequence is more likely to be stably maintained in the cell. Integration of the DNA constructs into the host chromosome may be performed according to conventional methods, *e.g.* by homologous or heterologous recombination.

Transformation of insect cells and production of heterologous polypeptides therein may be performed as described in US 4,745,051; US 4,879,236; US 5,155,037; US 5,162,222; and EP 397,485. The insect cell line used as the host may suitably be a Lepidoptera cell line, such as *Spodoptera frugiperda* cells or *Trichoplusia ni* cells (*cf.* US 5,077,214). Culture conditions may suitably be as described in, for instance, WO 89/01029 or WO 89/01028, or any of the aforementioned references.

The transformed or transfected host cell described above is then cultured in a suitable nutrient medium under conditions permitting expression of the *Plasmodium falciparum* VAR2CSA polypeptide after which all or part of the resulting peptide may be recovered from the culture. The medium used to culture the cells may be any conventional medium suitable for growing the host cells, such as minimal or complex media containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (*e.g.* in catalogues of the American Type Culture Collection). The *Plasmodium falciparum* VAR2CSA polypeptide produced by the cells may then be recovered from the culture medium by conventional procedures including separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaqueous components of the supernatant or filtrate by means of a salt, *e.g.* ammonium sulfate, purification by a variety of chromatographic procedures, *e.g.* ion exchange chromatography, gel filtration chromatography, affinity chromatography, or the like, dependent on the type of polypeptide in question.

Transgenic animal technology may be employed to produce the VAR2CSA polypeptides described herein. It is preferred to produce the proteins within the mammary glands of a host female mammal. Expression in the mammary gland and subsequent secretion of the protein of interest into the milk overcomes many difficulties encountered in isolating proteins from other sources. Milk is readily collected, available in large quantities, and biochemically well characterized. Furthermore, the major milk proteins are present in milk at high concentrations (typically from about 1 to 15 g/L).

From a commercial point of view, it is clearly preferable to use as the host a species that has a large milk yield. While smaller animals such as mice and rats can be used (and are preferred at the proof of principle stage), it is preferred to use livestock mammals including, but not limited to, pigs, goats, sheep and cattle. Sheep are particularly preferred due to such factors as the previous history of transgenesis in this species, milk yield, cost and the ready availability of equipment for collecting

sheep milk (see, for example, WO 88/00239 for a comparison of factors influencing the choice of host species). It is generally desirable to select a breed of host animal that has been bred for dairy use, such as East Friesland sheep, or to introduce dairy stock by breeding of the transgenic line at a later date. In any event, animals of known, good health status should be used.

To obtain expression in the mammary gland, a transcription promoter from a milk protein gene is used. Milk protein genes include those genes encoding caseins (see U.S. 5,304,489), beta lactoglobulin, a lactalbumin, and whey acidic protein. The beta lactoglobulin (BLG) promoter is preferred. In the case of the ovine beta lactoglobulin gene, a region of at least the proximal 406 bp of 5' flanking sequence of the gene will generally be used, although larger portions of the 5' flanking sequence, up to about 5 kbp, are preferred, such as a ~4.25 kbp DNA segment encompassing the 5' flanking promoter and non coding portion of the beta lactoglobulin gene (see Whitelaw *et al.*, Biochem. J. 286: 31 39 (1992)). Similar fragments of promoter DNA from other species are also suitable.

Other regions of the beta lactoglobulin gene may also be incorporated in constructs, as may genomic regions of the gene to be expressed. It is generally accepted in the art that constructs lacking introns, for example, express poorly in comparison with those that contain such DNA sequences (see Brinster *et al.*, Proc. Natl. Acad. Sci. USA 85: 836 840 (1988); Palmiter *et al.*, Proc. Natl. Acad. Sci. USA 88: 478 482 (1991); Whitelaw *et al.*, Transgenic Res. 1: 3 13 (1991); WO 89/01343; and WO 91/02318). In this regard, it is generally preferred, where possible, to use genomic sequences containing all or some of the native introns of a gene encoding the protein or polypeptide of interest, thus the further inclusion of at least some introns from, *e.g*, the beta lactoglobulin gene, is preferred. One such region is a DNA segment that provides for intron splicing and RNA polyadenylation from the 3' non coding region of the ovine beta lactoglobulin gene. When substituted for the natural 3' non coding sequences of a gene, this ovine beta lactoglobulin segment can both enhance and stabilize expression levels of the protein or polypeptide of interest. Within other embodiments, the region surrounding the initiation ATG of the VAR2CSA sequence is replaced with corresponding sequences from a milk specific protein gene. Such replacement provides a putative tissue specific initiation environment to enhance expression. It is convenient to replace the entire VAR2CSA pre pro and 5' non coding sequences with those of, for example, the BLG gene, although smaller regions may be replaced.

For expression of VAR2CSA polypeptides described herein in transgenic animals, a DNA segment encoding VAR2CSA is operably linked to additional DNA segments required for its expression to produce expression units. Such additional segments include the above mentioned promoter, as well as sequences that provide for termination of transcription and polyadenylation of mRNA. The expression units will further include a DNA segment encoding a secretory signal sequence operably linked to the segment encoding modified VAR2CSA. The secretory signal

sequence may be a native secretory signal sequence or may be that of another protein, such as a milk protein (see, for example, von Heijne, Nucl. Acids Res. 14: 4683-90 (1986); and Meade *et al.*, U.S. 4,873,316).

Construction of expression units for use in transgenic animals is conveniently carried out by inserting a VAR2CSA sequence into a plasmid or phage vector containing the additional DNA segments, although the expression unit may be constructed by essentially any sequence of ligations. It is particularly convenient to provide a vector containing a DNA segment encoding a milk protein and to replace the coding sequence for the milk protein with that of a VAR2CSA variant; thereby creating a gene fusion that includes the expression control sequences of the milk protein gene. In any event, cloning of the expression units in plasmids or other vectors facilitates the amplification of the VAR2CSA sequence. Amplification is conveniently carried out in bacterial (*e.g. E. coli*) host cells, thus the vectors will typically include an origin of replication and a selectable marker functional in bacterial host cells. The expression unit is then introduced into fertilized eggs (including early stage embryos) of the chosen host species. Introduction of heterologous DNA can be accomplished by one of several routes, including microinjection (*e.g.* U.S. Patent No. 4,873,191), retroviral infection (Jaenisch, Science 240: 1468-74 (1988)) or site directed integration using embryonic stem (ES) cells (reviewed by Bradley *et al.*, Biotechnology 10: 534-9 (1992)). The eggs are then implanted into the oviducts or uteri of pseudopregnant females and allowed to develop to term. Offspring carrying the introduced DNA in their germ line can pass the DNA on to their progeny in the normal, Mendelian fashion, allowing the development of transgenic herds. General procedures for producing transgenic animals are known in the art (see, for example, Hogan *et al.*, Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor Laboratory, 1986; Simons *et al.*, Biotechnology 6: 179-83 (1988); Wall *et al.*, Biol. Reprod. 32: 645 651 (1985); Buhler *et al.*, Biotechnology 8: 140-3 (1990); Ebert *et al.*, Biotechnology 9: 835-8 (1991); Krimpenfort *et al.*, Biotechnology 9: 844-7 (1991); Wall *et al.*, J. Cell. Biochem. 49: 113-20 (1992); U.S. 4,873,191; U.S. 4,873,316; WO 88/00239, WO 90/05188, WO 92/11757; and GB 87/00458). Techniques for introducing foreign DNA sequences into mammals and their germ cells were originally developed in the mouse (see, *e.g.*, Gordon *et al.*, Proc. Natl. Acad. Sci. USA 77: 7380-84 (1980); Gordon and Ruddle, Science 214: 1244-46 (1981); Palmiter and Brinster, Cell 41: 343-5 (1985); Brinster *et al.*, Proc. Natl. Acad. Sci. USA 82: 4438-42 (1985); and Hogan *et al.* (*ibid.*)). These techniques were subsequently adapted for use with larger animals, including livestock species (see, *e.g.*, WO 88/00239, WO 90/05188, and WO 92/11757; and Simons *et al.*, Biotechnology 6: 179-83 (1988)). To summarize, in the most efficient route used to date in the generation of transgenic mice or livestock, several hundred linear molecules of the DNA of interest are injected into one of the pro nuclei of a fertilized egg according to established techniques. Injection of DNA into the cytoplasm of a zygote can also be employed.

Production in transgenic plants may also be employed. Expression may be generalized or directed to a particular organ, such as a tuber (see, Hiatt, Nature 344:469 479 (1990); Edelbaum *et al.*, J. Interferon Res. 12:449 453 (1992); Sijmons *et al.*, Biotechnology 8:217-21 (1990); and EP 0 255 378).

The VAR2CSA polypeptides described herein may be recovered from cell culture medium or milk. The VAR2CSA polypeptides described herein may be purified by a variety of procedures known in the art including, but not limited to, chromatography (*e.g.*, ion exchange, affinity, hydrophobic, chromatofocusing, and size exclusion), electrophoretic procedures (*e.g.*, preparative isoelectric focusing (IEF), differential solubility (*e.g.*, ammonium sulfate precipitation), or extraction (see, *e.g.*, Protein Purification, J.-C. Janson and Lars Ryden, editors, VCH Publishers, New York, 1989). Preferably, they may be purified by affinity chromatography on an anti-VAR2CSA antibody column. Additional purification may be achieved by conventional chemical purification means, such as high performance liquid chromatography. Other methods of purification, including barium citrate precipitation, are known in the art, and may be applied to the purification of the VAR2CSA polypeptides described herein (see, for example, Scopes, R., Protein Purification, Springer-Verlag, N.Y., 1982).

For therapeutic purposes it is preferred that the VAR2CSA polypeptides described herein are substantially pure. Thus, in a preferred embodiment, the VAR2CSA polypeptides described herein are purified to at least about 90 to 95% homogeneity, preferably to at least about 98% homogeneity. Purity may be assessed by *e.g.* gel electrophoresis and amino-terminal amino acid sequencing.

LINKER MOIETY L^1

Provided are compounds of Formula IV:

$$T\text{–}L^1\text{–}P^1$$

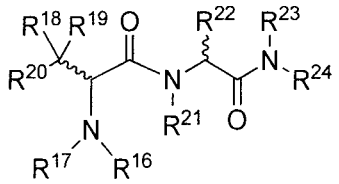
IV

wherein:

T is a targeting moiety comprising a VAR2CSA polypeptide;

L^1 is a linker, or L^1 is absent; and

P^1 is a monovalent radical of a compound of Formula XIV



XIV

wherein R^{16}, R^{17}, R^{18}, R^{19}, R^{20}, R^{21}, R^{22}, R^{23}, and R^{24} are as defined herein *supra* and *infra*.

The linker moiety L^1 is a bifunctional compound which can be used to link payload P^1 and targeting moiety T to form a conjugate compound, T-L^1-P^1. Such conjugates allow the selective delivery of drugs to target cells (*e.g.*, tumor cells). Linker moieties include a divalent substituent such as an alkyldiyl, an aryldiyl, a heteroaryldiyl, moieties such as: $-(CR_2)_nO(CR_2)_n-$, repeating units of alkyloxy (*e.g.*, polyethylenoxy, PEG, polymethyleneoxy) and alkylamino (*e.g.*, polyethyleneamino, Jeffamine™); and diacid ester and amides including succinate, succinamide, diglycolate, malonate, and caproamide. The compounds described herein can be prepared using a linker moiety having a reactive site for binding to the payload and the targeting moiety.

In some embodiments, L^1 has a reactive site which has an electrophilic group that is reactive to a nucleophilic group present on T. Useful nucleophilic groups on T include but are not limited to sulfhydryl, hydroxyl and amino groups. The heteroatom of the nucleophilic group of T is reactive to an electrophilic group on L^1 and forms a covalent bond to L^1. Useful electrophilic groups include, but are not limited to maleimide and haloacetamide groups. The nucleophilic group on T provide a convenient site for attachment to L^1.

In some embodiments, L^1 has a reactive site which has a nucleophilic group that is reactive to an electrophilic group present on the targeting moiety. Useful electrophilic groups on the targeting moiety include, but are not limited to, aldehyde and ketone carbonyl groups. The heteroatom of a nucleophilic group of L^1 can react with an electrophilic group on the targeting moiety and form a covalent bond to the targeting moiety. Useful nucleophilic groups on L^1 include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide. The electrophilic group on the targeting moiety provides a convenient site for attachment to L^1.

Carboxylic acid functional groups and chloroformate functional groups are also useful reactive sites for L^1 because they can react, for example, with an amino group of P^1 to form an amide linkage. Also useful as a reactive site is a carbonate functional group on L^1, such as but not limited to *p*-nitrophenyl carbonate, which can react, for example, with an amino group of P^1 to form a carbamate linkage.

It will be appreciated that any linker moieties taught in the prior art, and particularly those taught for use in the context of drug delivery, may be used in the current invention. In some embodiments, linkers with features well suited to a particular use, such as susceptibility to enzymatic cleavage or chemical cleavage within a cell of interest, may be used.

Without limiting the scope of the preceding statement, in one embodiment, L^1 comprises a linker moiety disclosed in WO 2012/113847. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 8,288,352. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 5,028,697. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 5,006,652. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 5,094,849. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 5,053,394. In another embodiment, L^1

comprises a linker moiety disclosed in U.S. 5,122,368. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 5,387,578. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 5,547,667. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 5,622,929. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 5,708,146. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 6,468,522. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 6,103,236. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 6,638,509. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 6,214,345. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 6,759,509. In another embodiment, L^1 comprises a linker moiety disclosed in WO 2007/103288. In another embodiment, L^1 comprises a linker moiety disclosed in WO 2008/083312. In another embodiment, L^1 comprises a linker moiety disclosed in WO 2003/068144. In another embodiment, L^1 comprises a linker moiety disclosed in WO 2004/016801. In another embodiment, L^1 comprises a linker moiety disclosed in WO 2009/134976. In another embodiment, L^1 comprises a linker moiety disclosed in WO 2009/134952. In another embodiment, L^1 comprises a linker moiety disclosed in WO 2009/134977. In another embodiment, L^1 comprises a linker moiety disclosed in WO 2002/08180. In another embodiment, L^1 comprises a linker moiety disclosed in WO 2004/043493. In another embodiment, L^1 comprises a linker moiety disclosed in WO 2007/018431. In another embodiment, L^1 comprises a linker moiety disclosed in WO 2003/026577. In another embodiment, L^1 comprises a linker moiety disclosed in WO 2005/077090. In another embodiment, L^1 comprises a linker moiety disclosed in WO 2005/082023. In another embodiment, L^1 comprises a linker moiety disclosed in WO 2007/011968. In another embodiment, L^1 comprises a linker moiety disclosed in WO 2007/038658. In another embodiment, L^1 comprises a linker moiety disclosed in WO 2007/059404. In another embodiment, L^1 comprises a linker moiety disclosed in WO 2006/110476. In another embodiment, L^1 comprises a linker moiety disclosed in WO 2005/112919. In another embodiment, L^1 comprises a linker moiety disclosed in WO 2008/103693. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 6,756,037. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 7,087,229. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 7,122,189. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 7,332,164. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 5,556,623. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 5,643,573. In another embodiment, L^1 comprises a linker moiety disclosed in U.S. 5,665,358. Linkers L^1 comprising a self-immolative component may also be used. For example, see U.S. Pat. No. 6,214,345. An example of a self-immolative component is p-aminobenzylcarbamoyl (PABC). Commercially available linkers may be used in the invention. For example, the commercially available cleavable linker sulfosuccinimidyl 6-[3′(2-pyridyldithio)-propionamido] hexanoate (sulfo-LC-SPDP: Thermo Pierce Cat# 21650) and Non-cleavable linker succinimidyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate (SMCC: Thermo Pierce Cat# 22360) may be used, as

demonstrated herein. See also, WO2012171020, WO2010138719, the range of commercially available linkers, for example, from Concortis http://www.concortis.com/home. See also Kim *et al.*, Bioconjugate Chemistry, 21 (8): 1513-1519 AUG 2010. See also EP2326349. See also copper-free click chemistry linkers, Angew. Chem. Int. Ed., 2010, 49, p. 9422-9425, ChemBioChem, 2011, 12, p. 1309-1312, http://www.synaffix.com/technology/.

In some embodiments, L^1 comprises: SPDP, SMCC, vcPABC, MCvcPABC, MTvc, ADvc, maleimide, NHS, biotin, streptavidin, NeutrAvidin, a glycoside, or a combination thereof.

In some embodiments, L^1 comprises SPDP.

In some embodiments, L^1 comprises SMCC.

In some embodiments, L^1 comprises vcPABC.

In some embodiments, L^1 comprises MCvcPABC.

In some embodiments, L^1 comprises MTvc.

In some embodiments, L^1 comprises ADvc.

In some embodiments, L^1 comprises maleimide.

In some embodiments, L^1 comprises NHS.

In some embodiments, L^1 comprises biotin.

In some embodiments, L^1 comprises streptavidin.

In some embodiments, L^1 comprises NeutrAvidin.

In some embodiments, L^1 comprises a glycoside.

In some embodiments, L^1 is absent.

PAYLOAD MOIETY P^1

Talpir, R. *et al.* (1994) Tetrahedron Lett. 35:4453-6, describe the naturally occurring compound hemiasterlin, a stable tripeptide obtained from marine sponges that causes microtubule depolymerization and mitotic arrest in cells. Hemisasterlin consists of unusual and highly congested amino acids, features thought to contribute to its activity. A number of groups have modified particular structural elements of hemiasterlin to evaluate structure-activity relationships and assess the activity of hemiasterlin analogs. See for example Zask *et al.*, Bioorganic & Medicinal Chemistry Letters, 14:4353-4358, 2004; Zask *et al.*, J Med Chem, 47:4774-4786, 2004; Yamashita *et al.*, Bioorganic & Medicinal Chemistry Letters, 14:5317-5322, 2004; PCT/GB96/00942; WO 2004/026293; WO96/33211; and U.S. 7,579,323.

Analogs of hemiasterlin with modifications in the "A-segment", or the amino terminal segment, have been described (see for example, Zask *et al.*, J Med Chem, 47:4774-4786, 2004; Yamashita *et al.*, Bioorganic & Medicinal Chemistry Letters, 14:5317-5322, 2004; U.S. 7,579,323). U.S. 7,579,323 discloses an analog of hemiasterlin, referred to as HTI-286, in which the indole moiety

is replaced by a phenyl group. HTI-286 exhibits potent anti-mitotic activity and has been assessed in clinical trials for the treatment of cancer (Ratain *et al.*, Proc Am Soc Clin Oncol, 22:129, 2003).

Analogs of hemiasterlin with modifications in the "D-segment", or the carboxy terminal segment, have also been reported (see, for example, WO 2004/026293; Zask *et al.*, Bioorganic & Medicinal Chemistry Letters, 14:4353-4358, 2004; Zask *et al.*, J Med Chem, 47:4774-4786, 2004). The majority of modifications at the carboxy terminus result in compounds with substantially decreased potency compared to parent carboxylic acids. See, for example, WO 2004/026293, particularly Table 12. Zask *et al.*,(J Med Chem, 47:4774-4786, 2004) also report that amide analogs prepared using simple cyclic and acyclic amines exhibit significantly reduced potency (reductions of one to three orders of magnitude). Among the few tolerated modifications, Zask *et al.*, (Bioorganic & Medicinal Chemistry Letters, 14:4353-4358, 2004) report that the addition of esterified cyclic amino acids at the carboxy-terminus yields tetrapeptide analogs with prolyl-like ester-containing termini, some of which exhibit potency comparable to parent compound in a tested cancer cell line.

While a wide variety of hemiasterlin analogs have been generated, many, including a wide variety of compounds with modifications at the carboxy terminus, exhibit reduced potency that limits utility in methods of medical treatment. However, certain hemiasterlin analogs modified by the addition of an *N*-acyl sulfonamide moiety at the carboxy terminus, such as those disclosed in International Application No. PCT/US14/29463 or U.S. Serial No. 14/213,504, demonstrate potent anticancer activity across a broad range of cancer cell lines.

Accordingly, provided are compounds of Formula IV:

$$T\text{–}L^1\text{–}P^1$$

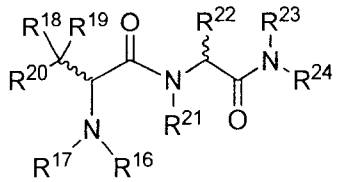
IV

wherein:

T is a targeting moiety comprising a VAR2CSA polypeptide;

L^1 is a linker, or L is absent; and

P^1 is a monovalent radical of a compound of Formula XIV:



XIV

wherein:

R^{16} and R^{17} are independently selected from the group consisting of: H and a saturated or unsaturated moiety having a linear, branched, or non-aromatic cyclic skeleton containing one to ten carbon atoms, and the carbon atoms are optionally substituted with: –OH, –I, –Br, –Cl, –F, –CN, $–CO_2H$, –CHO, –COSH, or $–NO_2$; or R^{17} and R^{20} are fused and form a ring;

R^{18} and R^{19} are independently selected from the group consisting of: H, R^{25}, and ArR^{25}–, or R^{18} and R^{19} are joined to form a ring;

R^{20} is selected from the group consisting of: H, R^{25}, ArR^{25}–, and Ar; or R^{20} and R^{17} are fused and form a ring;

R^{21} is selected from the group consisting of: H, R^{25}, and ArR^{25}–;

R^{22} and R^{23} are independently selected from the group consisting of: H, R^{25}, and ArR^{25}–;

R^{24} is: $–Y–(CO)NHSO_2–R^{26}$

R^{25} is defined as a saturated or unsaturated moiety having a linear, branched, or non-aromatic cyclic skeleton containing one to ten carbon atoms, zero to four nitrogen atoms, zero to four oxygen atoms, and zero to four sulfur atoms, and the carbon atoms are optionally substituted with: =O, =S, OH, $–OR^{28}$, $–O_2CR^{28}$, –SH, $–SR^{28}$, $–SOCR^{28}$, $–NH_2$, $–NHR^{28}$, $–N(R^{28})_2$, $–NHCOR^{28}$, $–NR^{28}COR^{28}$, –I, –Br, –Cl, –F, –CN, $–CO_2H$, $–CO_2R^{28}$, –CHO, $–COR^{28}$, $–CONH_2$, $–CONHR^{28}$, $–CON(R^{28})_2$, –COSH, $–COSR^{28}$, $-NO_2$, $-SO_3H$, $-SOR^{28}$, $-SO_2R^{28}$, wherein R^{28} is a linear, branched or cyclic, one to ten carbon saturated or unsaturated alkyl group;

the ring formed by joining R^{18} and R^{19} is a three to seven member non-aromatic cyclic skeleton within the definition of R^{25},

Y is defined as a moiety selected from the group consisting of: a linear, saturated or unsaturated, one to six carbon alkyl group, optionally substituted with R^{25}, ArR^{25}–, or X; and,

X is defined as a moiety selected from the group consisting of: –OH, $–OR^{25}$, =O, =S, $–O_2CR^{25}$, –SH, $–SR^{25}$, $–SOCR^{25}$, $–NH_2$, $–NHR^{25}$, $–N(R^{25})_2$, $–NHCOR^{25}$, $–NRCOR^{25}$, –I, –Br, –Cl, –F, –CN, $–CO_2H$, $–CO_2R^{25}$, –CHO, $–COR^{25}$, $–CONH_2$, $–CONHR^{25}$, $–CON(R^{25})_2$, –COSH, $–COSR^{25}$, $–NO_2$, $–SO_3H$, $–SOR^{25}$, and $–SO_2R^{25}$;

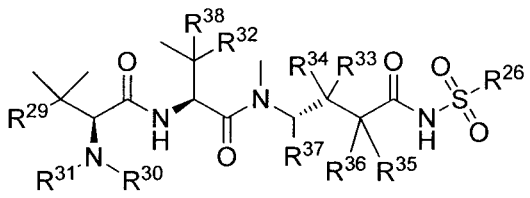
R^{26} is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl, $–COR^{27}$, $–CSR^{27}$, $–OR^{27}$, and $–NHR^{27}$, wherein each R^{27} is, independently, optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl or optionally substituted heteroaryl;

or a stereoisomer, prodrug or pharmaceutically acceptable salt thereof.

In a preferred embodiment, R^{26} is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl.

In one embodiment, Ar is an aromatic ring selected from the group consisting of: phenyl, naphthyl, anthracyl, pyrrolyl.

In some embodiments, P^1 is a monovalent radical of a compound of Formula XV:



XV

wherein:

R^{26} is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, $-COR^{27}$, $-CSR^{27}$, $-OR^{27}$, and $-NHR^{27}$, wherein each R^{27} is, independently, alkyl optionally substituted with halogen, –OH or –SH;

R^{29} is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl;

R^{30} is selected from the group consisting of H and C_{1-6} alkyl;

R^{31} is selected from the group consisting of H and C_{1-6} alkyl;

R^{32} and R^{38} are independently selected from the group consisting of H, C_{1-6} alkyl and –SH, with the proviso that R^{32} and R^{38} cannot both be H;

R^{33}, R^{34}, R^{35} and R^{36} are independently H and C_{1-6} alkyl, at least one of R^{33} and R^{34} is H; or R^{34} and R^{35} form a double bond, R^{33} is H, and R^{36} is H or C_{1-6} alkyl; and

R^{37} is selected from the group consisting of H and C_{1-6} alkyl;

or a stereoisomer, prodrug or pharmaceutically acceptable salt thereof.

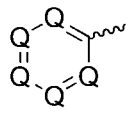
In a preferred embodiment, R^{26} is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl.

In a further embodiment, each optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl is, independently, optionally substituted with =O, =S, $-OH$, $-OR^{27}$, $-O_2CR^{27}$, $-SH$, $-SR^{27}$, $-SOCR^{27}$, $-NH_2$, $-N_3$, $-NHR^{27}$, $-N(R^{27})_2$, $-NHCOR^{27}$, $-NR^{27}COR^{27}$, –I, –Br, –Cl, –F, –CN, $-CO_2H$, $-CO_2R^{27}$, –CHO, $-COR^{27}$, $-CONH_2$, $-CONHR^{27}$, $-CON(R^{27})_2$, –COSH, $-COSR^{27}$, $-NO_2$, $-SO_3H$, $-SOR^{27}$ or $-SO_2R^{27}$wherein each R^{27} is, independently, alkyl optionally substituted with halogen, –OH or –SH.

In another further embodiment, each optionally substituted aryl and optionally substituted heteroaryl is, independently, selected from the group consisting of optionally substituted phenyl, optionally substituted naphthyl, optionally substituted anthracyl, optionally substituted phenanthryl, optionally substituted furyl, optionally substituted pyrrolyl, optionally substituted thiophenyl, optionally substituted benzofuryl, optionally substituted benzothiophenyl, optionally substituted

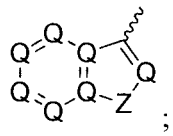
quinolinyl, optionally substituted isoquinolinyl, optionally substituted imidazolyl, optionally substituted thiazolyl, optionally substituted oxazolyl, and optionally substituted pyridinyl.

In another further embodiment, R^{29} is selected from one of the following structures XVI, XVII, XVIII, and XIX:



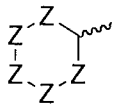
;

XVI



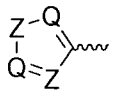
;

XVII



; and

XVIII



,

XIX

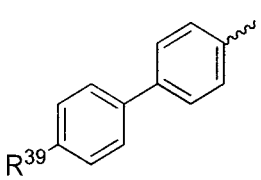
wherein:

Q is CR^{39} or N;

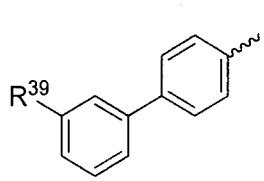
Z is $C(R^{39})_2$, NR^{39}, S, or O;

each R^{39} is, independently, selected from the group consisting of H, –OH, $–R^{27}$, $–OR^{27}$, $–O_2CR^{27}$, –SH, $–SR^{27}$, $–SOCR^{27}$, $–NH_2$, $–N_3$, $–NHR^{27}$, $–N(R^{27})_2$, $–NHCOR^{27}$, $–NR^{27}COR^{27}$, $–R^{27}NH_2$, –I, –Br, –Cl, –F, –CN, $–CO_2H$, $–CO_2R^{27}$, –CHO, $–COR^{27}$, $–CONH_2$, $–CONHR^{27}$, $–CON(R^{27})_2$, –COSH, $–COSR^{27}$, $–NO_2$, $–SO_3H$, $–SOR^{27}$, and $–SO_2R^{27}$, wherein each R^{27} is, independently, alkyl optionally substituted with halogen, –OH or –SH.

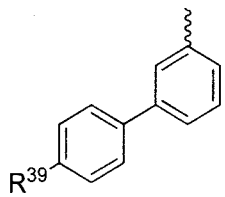
In another further embodiment, R^{29} is selected from the group consisting of:



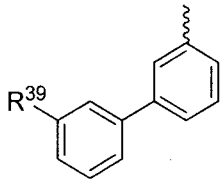
;



;

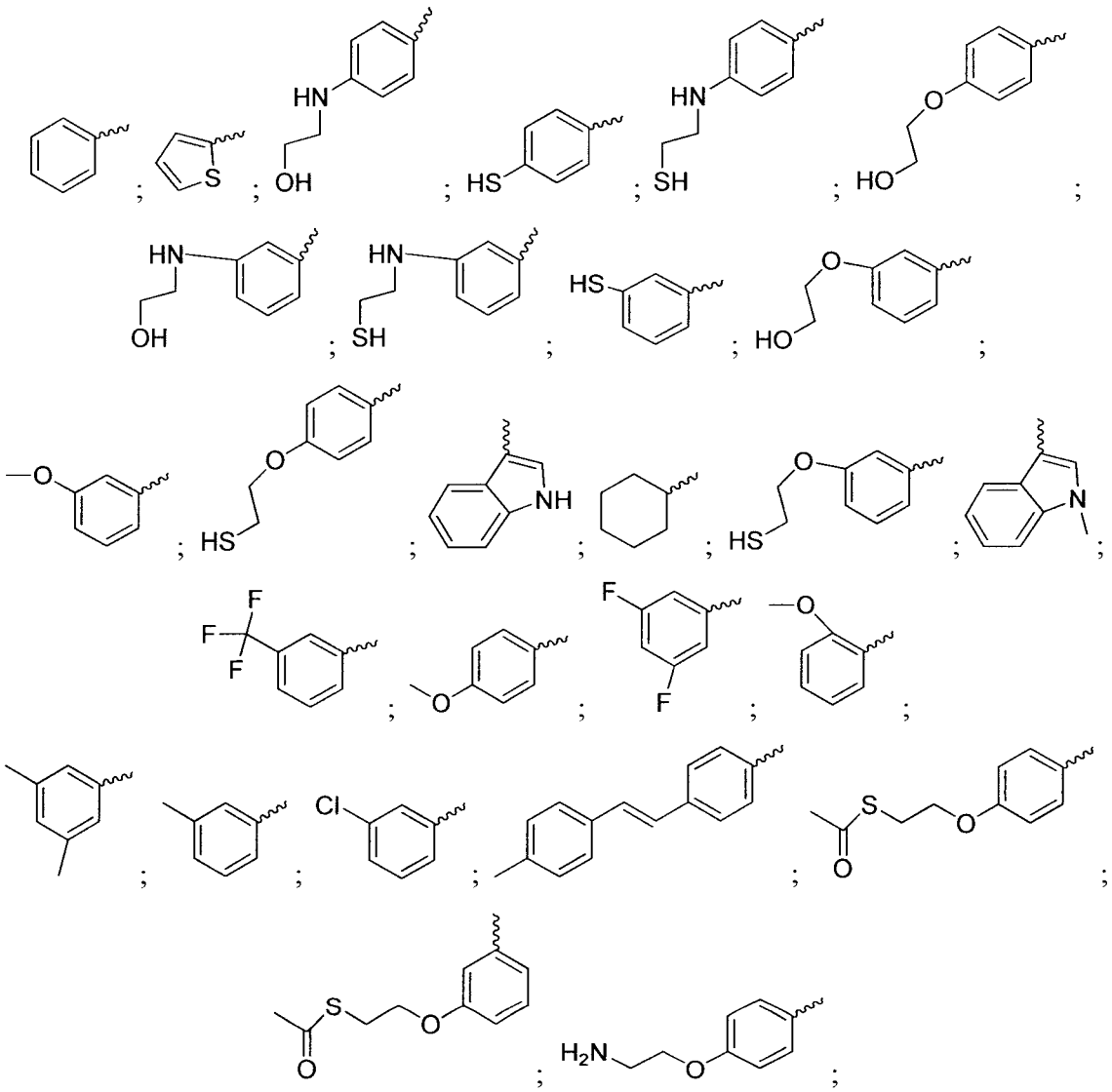


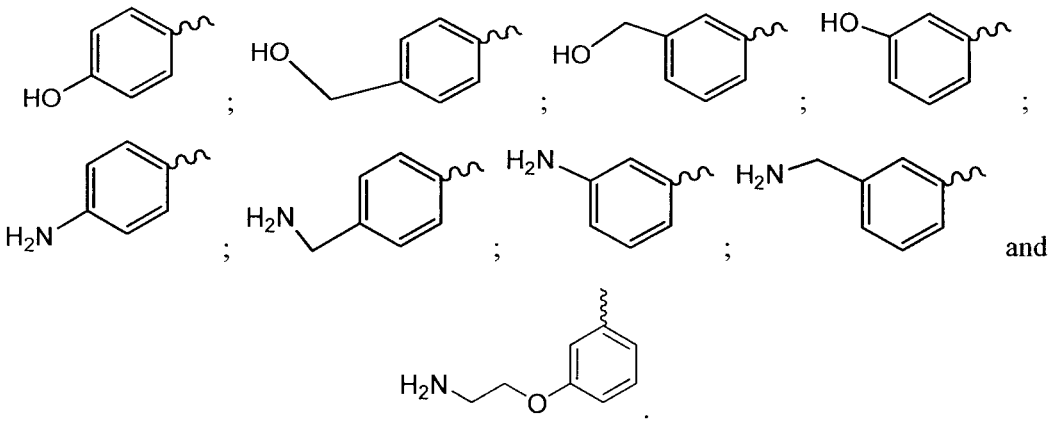
; and



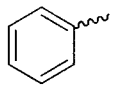
wherein each R^{39} is, independently, selected from the group consisting of H, –OH, $–R^{27}$, $–OR^{27}$, $-O_2CR^{27}$, –SH, $–SR^{27}$, $–SOCR^{27}$, $–NH_2$, $–N_3$, $–NHR^{27}$, $–N(R^{27})_2$, $–NHCOR^{27}$, $–NR^{27}COR^{27}$, $–R^{27}NH_2$, –I, –Br, –Cl, –F, –CN, $–CO_2H$, $–CO_2R^{27}$, –CHO, $–COR^{27}$, $–CONH_2$, $–CONHR^{27}$, $–CON(R^{27})_2$, –COSH, $–COSR^{27}$, $–NO_2$, $–SO_3H$, $–SOR^{27}$, and $–SO_2R^{27}$, wherein each R^{27} is, independently, alkyl optionally substituted with halogen, –OH or –SH.

In another further embodiment, R^{29} is selected from the group consisting of:





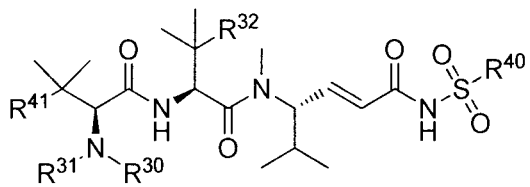
In another further embodiment, R^{29} is:



In another further embodiment, R^{30}, R^{31}, R^{32}, and R^{38} are each methyl.

In another further embodiment, R^{30} is H, R^{31} is methyl, R^{32} is methyl, and R^{38} is methyl.

In some embodiments, P^1 is a monovalent radical of a compound of Formula XX:



XX

wherein:

R^{40} is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, $-COR^{27}$, $-CSR^{27}$, $-OR^{27}$, and $-NHR^{27}$, wherein each R^{27} is, independently, optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl;

R^{41} is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl;

R^{30} is selected from the group consisting of H and C_{1-6} alkyl;

R^{31} is selected from the group consisting of H and C_{1-6} alkyl; and

R^{32} is selected from the group consisting of C_{1-6} alkyl and –SH,

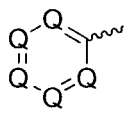
or a stereoisomer, prodrug or pharmaceutically acceptable salt thereof.

In a preferred embodiment, R^{40} is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl.

In a further embodiment, each optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl is, independently, optionally substituted with =O, =S, –OH, $–OR^{42}$, $–O_2CR^{42}$, –SH, $–SR^{42}$, $–SOCR^{42}$, $–NH_2$, $–N_3$, $–NHR^{42}$, $–N(R^{42})_2$, $–NHCOR^{42}$, $–NR^{42}COR^{42}$, –I, –Br, –Cl, –F, –CN, $–CO_2H$, $–CO_2R^{42}$, –CHO, $–COR^{42}$, $–CONH_2$, $–CONHR^{42}$, $–CON(R^{42})_2$, –COSH, $–COSR^{42}$, $–NO_2$, $–SO_3H$, $–SOR^{42}$ or $–SO_2R^{42}$, wherein each R^{42} is, independently, alkyl optionally substituted with halogen, –OH or –SH.

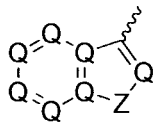
In another further embodiment, each optionally substituted aryl and optionally substituted heteroaryl is, independently, selected from the group consisting of optionally substituted phenyl, optionally substituted naphthyl, optionally substituted anthracyl, optionally substituted phenanthryl, optionally substituted furyl, optionally substituted pyrrolyl, optionally substituted thiophenyl, optionally substituted benzofuryl, optionally substituted benzothiophenyl, optionally substituted quinolinyl, optionally substituted isoquinolinyl, optionally substituted imidazolyl, optionally substituted thiazolyl, optionally substituted oxazolyl, and optionally substituted pyridinyl.

In another further embodiment, R^{41} is selected from one of the following structures XVI, XVII, XVIII, and XIX:



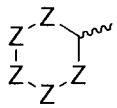
;

XVI



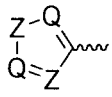
;

XVII



; and

XVIII



,

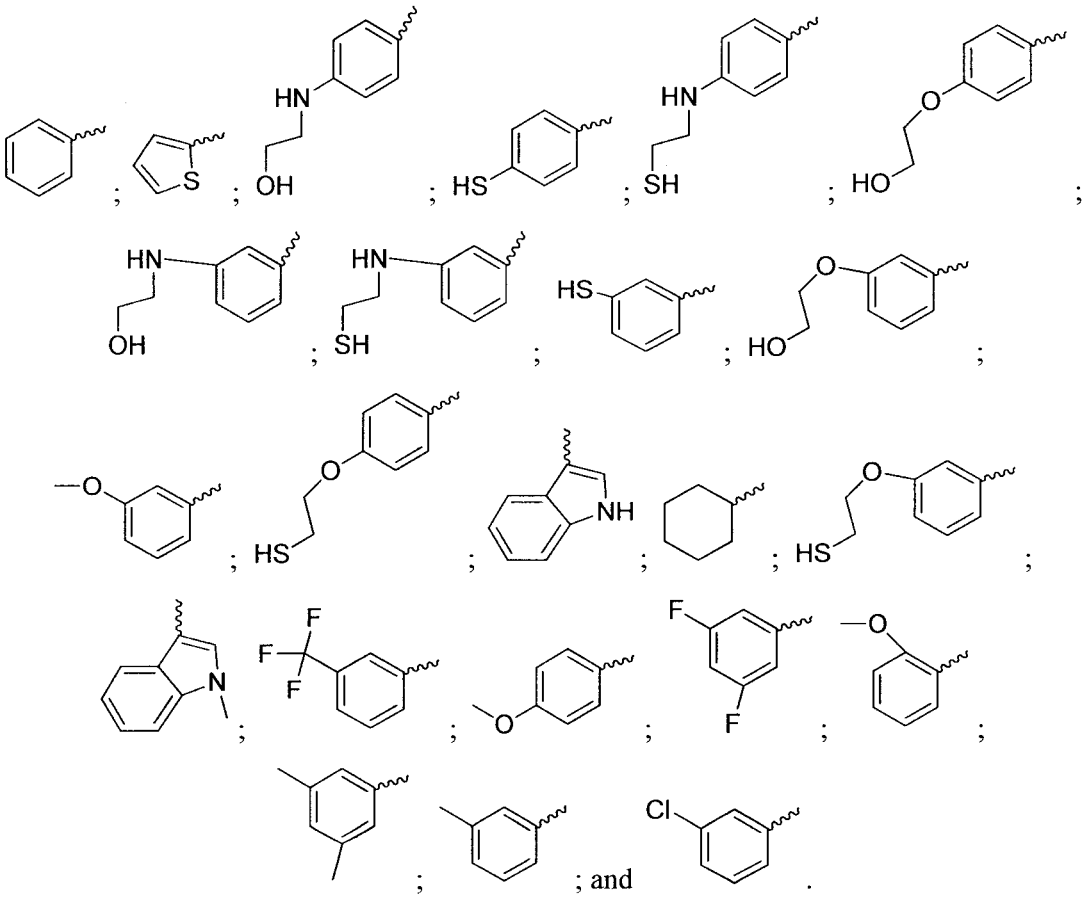
wherein:

Q is CR^{43} or N;

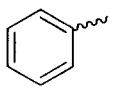
Z is $C(R^{43})_2$, NR^{43}, S, or O;

each R^{43} is, independently, selected from the group consisting of H, -OH, $-OR^{42}$, $-O_2CR^{42}$, -SH, $-SR^{42}$, $-SOCR^{42}$, $-NH_2$, $-N_3$, $-NHR^{42}$, $-N(R^{42})_2$, $-NHCOR^{42}$, $-NR^{42}COR^{42}$, -I, -Br, -Cl, -F, -CN, $-CO_2H$, $-CO_2R^{42}$, -CHO, $-COR^{42}$, $-CONH_2$, $-CONHR^{42}$, $-CON(R^{42})_2$, -COSH, $-COSR^{42}$, $-NO_2$, $-SO_3H$, $-SOR^{42}$, and $-SO_2R^{42}$, wherein each R^{42} is, independently, alkyl optionally substituted with halogen, -OH or -SH.

In another further embodiment, R^{41} is selected from the group consisting of:



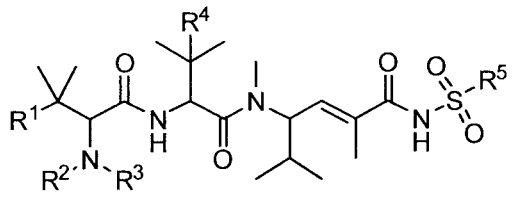
In another further embodiment, R^{41} is:



In another further embodiment, R^{30}, R^{31} and R^{32} are each methyl.

In another further embodiment, R^{30} is H, R^{31} is methyl, and R^{32} is methyl.

In some embodiments, P^1 is a monovalent radical of a compound of Formula II:



II

wherein:

R^1 is selected from: aryl, C_3-C_7 cycloalkyl, and heteroaryl, each of which is optionally substituted with one or more substituents selected from: C_1-C_4 acylthio, C_2-C_4 alkenyl, C_1-C_4 alkyl, C_1-C_4 alkylamino, C_1-C_4 alkoxy, amino, amino-C_1-C_4 alkyl, halo, C_1-C_4 haloalkyl, hydroxyl, hydroxy-C_1-C_4 alkyl, and thio, wherein C_2-C_4 alkenyl, C_1-C_4 alkylamino and C_1-C_4 alkoxy are further optionally substituted with one substituent selected from C_1-C_4 alkylaryl, hydroxyl, and thio;

R^2 and R^3 are each independently selected from: H and C_1-C_6 alkyl;

R^4 is selected from the group consisting of C_1-C_6 alkyl and thio; and

R^5 is selected from: C_1-C_6 alkyl, aryl, aryl-C_1-C_6 alkyl, C_3-C_7 cycloalkyl, heteroaryl, and heterocyclyl, each optionally substituted with one or more substituents selected from: C_1-C_6 alkoxy, C_1-C_6 alkoxycarbonyl, C_1-C_6 alkyl, C_1-C_6 alkylamino, amino, amino-C_1-C_6 alkyl, amino-aryl, amino-C_3-C_7 cycloalkyl, aryl, carboxamide, carboxyl, C_3-C_7 cycloalkyl, cyano, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, halo, hydroxyl, nitro, thio, and thio-C_1-C_6 alkyl; and

In some embodiments, R^1 is selected from: is selected from: H, aryl, C_3-C_7 cycloalkyl, and heteroaryl, each of which is optionally substituted with one or more substituents selected from: C_1-C_4 acylthio, C_2-C_4 alkenyl, C_1-C_4 alkyl, C_1-C_4 alkylamino, C_1-C_4 alkoxy, amino, amino-C_1-C_4 alkyl, halo, C_1-C_4 haloalkyl, hydroxyl, hydroxy-C_1-C_4 alkyl, and thio, wherein C_2-C_4 alkenyl, C_1-C_4 alkylamino and C_1-C_4 alkoxy are further optionally substituted with one substituent selected from *p*-tolyl, hydroxyl, and thio.

In some embodiments, R^1 is selected from: H, aryl, C_3-C_7 cycloalkyl, and heteroaryl, each of which is optionally substituted with one or more substituents selected from: (2-hydroxyethyl)amino, (2-mercaptoethyl)amino, 2-(acetylthio)ethoxy, 2-aminoethoxy, 2-hydroxyethoxy, 2-mercaptoethoxy, 3-methoxy, 4-methylstyryl, amino, aminomethyl, chloro, fluoro, hydroxyl, hydroxymethyl, methyl, thio, trifluoromethyl.

In some embodiments, R^1 is selected from: H, cyclohexyl, 1*H*-indol-3-yl, phenyl, and thien-2-yl each of which is optionally substituted with one or more substituents selected from: (2-hydroxyethyl)amino, (2-mercaptoethyl)amino, 2-(acetylthio)ethoxy, 2-aminoethoxy, 2-hydroxyethoxy, 2-mercaptoethoxy, 3-methoxy, 4-methylstyryl, amino, aminomethyl, chloro, fluoro, hydroxyl, hydroxymethyl, methyl, thio, and trifluoromethyl.

In some embodiments, R^1 is selected from: H, 1*H*-indol-3-yl, 1-methyl-1*H*-indol-3-yl, 2-methoxyphenyl, 3-((2-hydroxyethyl)amino)phenyl, 3-((2-mercaptoethyl)amino)phenyl, 3-(2-

(acetylthio)ethoxy)phenyl, 3-(2-hydroxyethoxy)phenyl, 3-(2-mercaptoethoxy)phenyl, 3-(4-methylstyryl)phenyl, 3-(aminomethyl)phenyl, 3-(hydroxymethyl)phenyl, 3-hydroxyphenyl, 3,5-difluorophenyl, 3,5-dimethylphenyl, 3-aminophenyl, 3-chlorophenyl, 3-mercaptophenyl, 3-methoxyphenyl, 3-trifluoromethylphenyl, 4-((2-hydroxyethyl)amino)phenyl, 4-((2-mercaptoethyl)amino)phenyl, 4-(2-(acetylthio)ethoxy)phenyl, 4-(2-aminoethoxy)phenyl, 4-(2-hydroxyethoxy)phenyl, 4-(2-mercaptoethoxy)phenyl, 4-(aminomethyl)phenyl, 4-(hydroxymethyl)phenyl, 4-aminophenyl, 4-hydroxyphenyl, 4-mercaptophenyl, 4-methoxyphenyl, cyclohexyl, thien-2-yl, *m*-tolyl, and phenyl.

In some embodiments, R^1 is selected from: H, 1*H*-indol-3-yl, 1-methyl-1*H*-indol-3-yl, 2-methoxyphenyl, 3-((2-hydroxyethyl)amino)phenyl, 3-((2-mercaptoethyl)amino)phenyl, 3-(2-hydroxyethoxy)phenyl, 3-(2-mercaptoethoxy)phenyl, 3,5-difluorophenyl, 3,5-dimethylphenyl, 3-chlorophenyl, 3-mercaptophenyl, 3-methoxyphenyl, 3-trifluoromethylphenyl, 4-((2-hydroxyethyl)amino)phenyl, 4-((2-mercaptoethyl)amino)phenyl, 4-4-(2-hydroxyethoxy)phenyl, 4-(2-mercaptoethoxy)phenyl, 4-mercaptophenyl, 4-methoxyphenyl, cyclohexyl, thien-2-yl, *m*-tolyl, and phenyl.

In some embodiments, R^1 is phenyl.

In some embodiments, R^2 is H.

In some embodiments, R^2 is methyl.

In some embodiments, R^3 is methyl.

In some embodiments, R^4 is methyl.

In some embodiments, R^5 is selected from: C_1-C_6 alkyl, aryl, aryl-C_1-C_6 alkyl, C_3-C_7 cycloalkyl, heteroaryl, and heterocyclyl, each optionally substituted with one or more substituents selected from: 1-aminocyclopropyl, 4-aminophenyl, amino, aminomethyl, bromo, *tert*-butyl, carboxamide, carboxyl, chloro, cyano, cyclopentyl, ethyl, fluoro, hydroxy, isopropyl, methoxy, methyl, nitro, phenyl, pyridin-3-yl, thio, thiomethyl, trifluoromethoxy, and trifluoromethyl.

In some embodiments, R^5 is selected from: 5,6,7,8-tetrahydronaphthalen-1-yl, benzyl, cyclohexyl, ethyl, hexan-2-yl, methyl, naphthalen-2-yl, piperidin-1-yl, phenyl, propyl, pyridin-3-yl, and thien-2-yl, each optionally substituted with one or more substituents selected from: 1-aminocyclopropyl, 4-aminophenyl, amino, aminomethyl, bromo, *tert*-butyl, carboxamide, carboxyl, chloro, cyano, cyclopentyl, ethyl, fluoro, hydroxy, isopropyl, methoxy, methyl, nitro, phenyl, pyridin-3-yl, thio, thiomethyl, trifluoromethoxy, and trifluoromethyl.

In some embodiments, R^5 is selected from: 4-aminobenzyl, 4-(aminomethyl)benzyl, 4-(aminomethyl)phenyl, 4-aminophenyl, benzyl, 3-mercaptopropyl, 2-mercaptoethyl, 4-(mercaptomethyl)phenyl, *p*-tolyl, methyl, 2,4,6-trimethylphenyl, 4-(trifluoromethoxy)phenyl, 2,4,6-triisopropylphenyl, 4-*tert*-butylphenyl, 4-chlorophenyl, 3-cyanophenyl, 2-nitrophenyl, 4-methoxy-2-nitrophenyl, 4-aminocarbonyl-2-nitrophenyl, 4-methoxyphenyl, 4-aminophenyl, phenyl, 2-

fluorobenzyl, piperidin-1-yl, *o*-tolyl, 4-bromophenyl, naphthalen-2-yl, 4-methoxycarbonyphenyl, 2-(trifluoromethyl)benzyl, hexan-2-yl, 2-methoxyethyl, cyclopentylmethyl, cyclohexyl, pyridin-3-ylmethyl, 4-carboxyphenyl, 3-aminophenyl, pyridin-3-yl, thien-2-yl, 4-hydroxyphenyl, 4-(1-aminocyclopropyl)benzyl, 4-(1-aminocyclopropyl)phenyl, 2-methylbenzyl, 4-nitrobenzyl, 4-chlorobenzyl, phenethyl, 4-bromobenzyl, 4-cyanobenzyl, 3-nitrobenzyl, 4-*tert*-butylbenzyl, 2-nitrobenzyl, 4-nitrophenethyl, 2-chloro-3-methoxycarbonylphenyl, 2-aminophenyl, [1,1'-biphenyl]-4-yl, 4'-amino-[1,1'-biphenyl]-4-yl, 4-fluorobenzyl, 3-(trifluoromethyl)benzyl, 3-(trifluoromethoxy)benzyl, 3,4-dichlorobenzyl, 2-cyanobenzyl, 3-chlorobenzyl, 4-amino-2-ethylphenyl, 4-amino-3-(trifluoromethoxy)phenyl, 4-amino-2,3-dimethylphenyl, 4-amino-5,6,7,8-tetrahydronaphthalen-1-yl, 4-amino-3-methylphenyl, 4-amino-3-fluorophenyl, 4-amino-3-ethylphenyl, and 4-amino-3-(trifluoromethyl)phenyl.

In some embodiments, R^5 is selected from: aryl and aryl-C_1-C_6 alkyl, each optionally substituted with one or more substituents selected from: amino and amino-C_1-C_6 alkyl.

In some embodiments, R^5 is selected from: 4-aminobenzyl, 4-(aminomethyl)benzyl, 4-(aminomethyl)phenyl, 4-aminophenyl, and benzyl.

In some embodiments, R^5 is 4-aminobenzyl.

In some embodiments, R^5 is 4-(aminomethyl)benzyl.

In some embodiments, R^5 is 4-(aminomethyl)phenyl.

In some embodiments, R^5 is 4-aminophenyl.

In some embodiments, wherein R^5 is benzyl.

In some embodiments P^1 is a monovalent radical of a compound disclosed in International Application No. PCT/US14/29463 or U.S. Serial No. 14/213,504.

It will be recognized by the artisan of reasonable skill that compounds of Formula XIV may be appropriately modified to facilitate a conjugation reaction with L^1, or if L^1 is not present, with T, and formation of a conjugate T–L^1–P^1 or T–P^1. Any point of attachment on P^1 may be used. In some embodiments, the C-terminus of P^1 forms the point of attachment in a T–L^1–P^1 conjugate. In another embodiment, the *N*-terminus of P^1 forms the point of attachment in a T–L^1–P^1 conjugate. In another embodiment, a side chain of P^1 forms the point of attachment in a T–L^1–P^1 conjugate.

In some embodiments, P^1 is a microtubule disrupting peptide toxin that covalently linked to L^1 through the side chain of the N-terminal amino acid of P^1, or if L^1 is not present, P^1 is covalently linked to T through the side chain of the N-terminal amino acid of P^1. In one embodiment, the microtubule disrupting peptide toxin is hemiasterlin or an analog thereof and the toxin is covalently linked in the conjugate through the indole moiety within the side chain of the N-terminal amino acid of the toxin peptide. In another embodiment, the microtubule disrupting peptide toxin is HTI-286 or an analog thereof and the toxin is covalently linked in the conjugate through the phenyl group within the side chain of the N-terminal amino acid of the toxin peptide. In one embodiment, the microtubule

disrupting peptide toxin is tubulysin or an analog thereof and the toxin is covalently linked in the conjugate through the indole moiety within the side chain of the N-terminal amino acid of the toxin peptide. In one embodiment, the microtubule disrupting peptide toxin is auristatin or an analog thereof and the toxin is covalently linked in the conjugate through the indole moiety within the side chain of the N-terminal amino acid of the toxin peptide. In one embodiment, the microtubule disrupting peptide toxin is a compound having structure XIV, XV, or XX .

In some embodiments, the compound $T–L^1–P^1$ has anti-mitotic activity and the following structure (XXI):

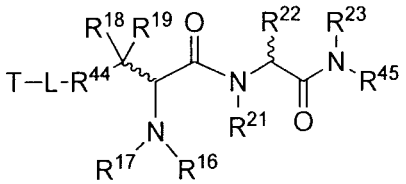
$$T–L^1–PT$$

XXI

wherein T is a targeting moiety as described herein, L is an optional linker as described herein, and PT is a microtubule disrupting peptide toxin that covalently linked to L^1 through the side chain of the N-terminal amino acid of PT, or if L is not present, PT is covalently linked to T through the side chain of the N-terminal amino acid of PT.

In one embodiment, VAR2CSA-drug conjugates comprising microtubule disrupting peptide toxins that are linked to the conjugate through the side chain of the N-terminal amino acid are provided.

In one embodiment, $T–L^1–PT$ has the following structure (XXII):



XXII

wherein,

R^{16} and R^{17} are independently selected from the group consisting of: H and a saturated or unsaturated moiety having a linear, branched, or non-aromatic cyclic skeleton containing one to ten carbon atoms, and the carbon atoms are optionally substituted with: –OH, –I, –Br, –Cl, –F, –CN, $–CO_2H$, –CHO, –COSH, or $–NO_2$;

R^{18} and R^{19} are independently selected from the group consisting of: H, R, $ArR^{25}–$, or R^{18} and R^{19} are joined to form a ring;

R^{44} is selected from the group consisting of: H, R^{46}, $ArR^{25}–$, $Ar–R^{25}–Ar$, $R^{25}–Ar–Ar$, $Ar–Ar–R^{25}–$, and Ar, wherein each R^{25} and each Ar may be substituted, and zero to ten heteroatoms may replace carbon atoms in the chain, for example O or S or N may be incorporated into the carbon chain; and wherein R^{46} is $–(CH_2–CH_2–O)_m–$, wherein m is an integer from one to fifteen;

R^{21} is selected from the group consisting of: H, R^{25}, and $ArR^{25}–$;

R^{22} and R^{23} are independently selected from the group consisting of: H, R, and $ArR^{25}–$; and

R^{45} is Z–C(O)–Y–; –Z–C(O)NHS(O)$_2$–R^{26}; or –Y–C(O)NHS(O)$_2$–R^{26};

wherein,

Z is defined as a moiety selected from the group consisting of: –OH, –OR^{25}; –SH; –SR^{25}; -NH_2; –$NR^{25}CH(R^{47})COOH$; and –$NHCH(R^{47})COOH$, wherein R^{47} is a moiety having the formula: R^{25}, or –$(CH_2)_nNR^{48}R^{49}$, wherein n = 1-4 and R^{48} and R^{49} are independently selected from the group consisting of: H; R^{25}; and –C(NH)(NH_2);

Y is defined as a moiety selected from the group consisting of: a linear, saturated or unsaturated, one to six carbon alkyl group, optionally substituted with R^{25}, ArR^{25}–, or X; and,

X is defined as a moiety selected from the group consisting of: –OH, –OR^{25}, =O, =S, –O_2CR^{25}, –SH, –SR^{25}, –$SOCR^{25}$, –NH_2, –NHR^{25}, –$N(R^{25})_2$, –$NHCOR^{25}$, –$NRCOR^{25}$, –I, –Br, –Cl, –F, –CN, –CO_2H, –CO_2R^{25}, –CHO, –COR^{25}, –$CONH_2$, –$CONHR^{25}$, –$CON(R^{25})_2$, –COSH, –$COSR^{25}$, –NO_2, –SO_3H, –SOR^{25}, and –SO_2R^{25};

R^{26} is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl, COR^{27}, –CSR^{27}, –OR^{27}, and –NHR^{27}, wherein each R^{27} is, independently, optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl or optionally substituted heteroaryl.

R^{25} is defined as a saturated or unsaturated moiety having a linear, branched, or non-aromatic cyclic skeleton containing one to ten carbon atoms, zero to four nitrogen atoms, zero to four oxygen atoms, and zero to four sulfur atoms, and the carbon atoms are optionally substituted with: =O, =S, OH, -OR^{28}, -O_2CR^{28}, -SH, -SR^{28}, -$SOCR^{28}$, -NH_2, -NHR^{28}, -$N(R^{28})_2$, -$NHCOR^{28}$, -$NR^{28}COR^{28}$, -I, -Br, -Cl, -F, -CN, -CO_2H, -CO_2R^{28}, -CHO, -COR^{28}, -$CONH_2$, -$CONHR^{28}$, -$CON(R^{28})_2$, -COSH, -$COSR^{28}$, -NO_2, -SO_3H, -SOR^{28}, -SO_2R^{28}, wherein R^{28} is a linear, branched or cyclic, one to ten carbon saturated or unsaturated alkyl group;

the ring formed by joining R^{18} and R^{19} is a three to seven member non-aromatic cyclic skeleton within the definition of R^{25},

Y is defined as a moiety selected from the group consisting of: a linear, saturated or unsaturated, one to six carbon alkyl group, optionally substituted with R, ArR^{25}–, or X; and,

X is defined as a moiety selected from the group consisting of: –OH, –OR, =O, =S, –O_2CR^{25}, –SH, –SR^{25}, –$SOCR^{25}$, –NH_2, –NHR^{25}, –$N(R^{25})_2$, –$NHCOR^{25}$, –$NRCOR^{25}$, –I, –Br, –Cl, –F, –CN, –CO_2H, –CO_2R^{25}, –CHO, –COR^{25}, –$CONH_2$, –$CONHR^{25}$, –$CON(R^{25})_2$, –COSH, –$COSR^{25}$, –NO_2, –SO_3H, –SOR^{25}, and –SO_2R^{25};

or a stereoisomer, prodrug or pharmaceutically acceptable salt thereof.

In a preferred embodiment, R^{26} is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl.

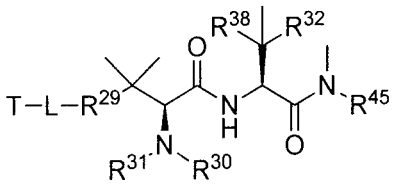
In one embodiment, Ar is an aromatic ring selected from the group consisting of: phenyl, naphthyl, anthracyl, pyrrolyl.

In one embodiment, R^{45} is Z–C(O)–Y–, wherein Z and Y are defined as above.

In one embodiment, R_{45} is –Z–C(O)NHS(O)$_2$–R^{26}, wherein Z and R^{26} are defined as above.

In one embodiment, R_{45} is –Y–C(O)NHS(O)$_2$–R^{26}, wherein Y and R^{26} are defined as above.

In another embodiment, T–L^1–PT has the following structure (XXIII):



XXIII

wherein,

R^{29} is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl;

R^{30} is selected from the group consisting of H and $C_{1\text{-}6}$ alkyl;

R^{31} is selected from the group consisting of H and $C_{1\text{-}6}$ alkyl;

R^{32} and R^{38} are independently selected from the group consisting of H, $C_{1\text{-}6}$ alkyl and -SH, with the proviso that both the R^{32} and R^{38} substituents cannot be H;

R^{45} is Z–C(O)–Y–; –Z–C(O)NHS(O)$_2$–R^{26}; or –Y–C(O)NHS(O)$_2$–R^{26};

wherein Y and R^{26} are defined as above;

Z is defined as a moiety selected from the group consisting of: –OH, –OR; –SH; –SR^{25}; –NH_2; –NR^{25}CH(R^{47})COOH; and –NHCH(R^{47})COOH, wherein R^{47} is a moiety having the formula: R^{25}, or –$(CH_2)_n$$NR^{48}R^{49}$, wherein n = 1-4 and R^{48} and R^{49} are independently selected from the group consisting of: H; R^{25}; and –C(NH)(NH_2),

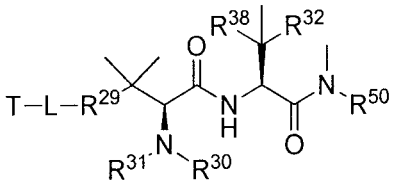
R^{25} is defined as a saturated or unsaturated moiety having a linear, branched, or non-aromatic cyclic skeleton containing one to ten carbon atoms, zero to four nitrogen atoms, zero to four oxygen atoms, and zero to four sulfur atoms, and the carbon atoms are optionally substituted with: =O, =S, OH, -OR^{28}, -O_2CR^{28}, -SH, -SR^{28}, -$SOCR^{28}$, -NH_2, -NHR^{28}, -$N(R^{28})_2$, -$NHCOR^{28}$, -$NR^{28}COR^{28}$, -I, -Br, -Cl, -F, -CN, -CO_2H, -CO_2R^{28}, -CHO, -COR^{28}, -$CONH_2$, -$CONHR^{28}$, -$CON(R^{28})_2$, -COSH, -$COSR^{28}$, -NO_2, -SO_3H, -SOR^{28}, -SO_2R^{28}, wherein R^{28} is a linear, branched or cyclic, one to ten carbon saturated or unsaturated alkyl group;

Y is defined as a moiety selected from the group consisting of: a linear, saturated or unsaturated, one to six carbon alkyl group, optionally substituted with R^{25}, ArR^{25}–, or X; and,

X is defined as a moiety selected from the group consisting of: –OH, $–OR^{25}$, =O, =S, $–O_2CR^{25}$, –SH, $–SR^{25}$, $–SOCR^{25}$, $–NH_2$, $–NHR^{25}$, $–N(R)_2$, $–NHCOR^{25}$, $–NRCOR^{25}$, –I, –Br, –Cl, –F, –CN, $–CO_2H$, $–CO_2R^{25}$, –CHO, $–COR^{25}$, $–CONH_2$, $–CONHR^{25}$, $–CON(R^{25})_2$, –COSH, $–COSR^{25}$, $–NO_2$, $–SO_3H$, $–SOR^{25}$, and $–SO_2R^{25}$;

or a stereoisomer, prodrug or pharmaceutically acceptable salt thereof.

In another embodiment, $T–L^1–PT$ has the following structure (XXIV):



XXIV

wherein,

R^{29} is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl;

R^{30} is selected from the group consisting of H and C_{1-6} alkyl;

R^{31} is selected from the group consisting of H and C_{1-6} alkyl;

R^{32} and R^{38} are independently selected from the group consisting of H, C_{1-6} alkyl and -SH, with the proviso that both the R^{32} and R^{38} substituents cannot be H;

R^{50} is Z–C(O)–Y–, wherein Z is as defined above,

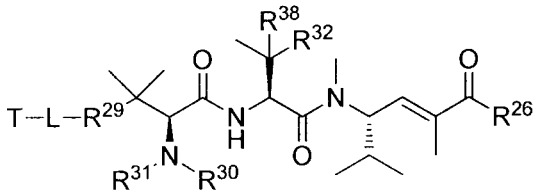
R^{25} is defined as a saturated or unsaturated moiety having a linear, branched, or non-aromatic cyclic skeleton containing one to ten carbon atoms, zero to four nitrogen atoms, zero to four oxygen atoms, and zero to four sulfur atoms, and the carbon atoms are optionally substituted with: =O, =S, OH, $–OR^{28}$, $–O_2CR^{28}$, –SH, $–SR^{28}$, $–SOCR^{28}$, $–NH_2$, $–NHR^{28}$, $–N(R^{28})_2$, $–NHCOR^{28}$, $–NR^{28}COR^{28}$, –I, –Br, –Cl, –F, –CN, $–CO_2H$, $–CO_2R^{28}$, –CHO, $–COR^{28}$, $–CONH_2$, $–CONHR^{28}$, $–CON(R^{28})_2$, –COSH, $–COSR^{28}$, $–NO_2$, $–SO_3H$, $–SOR^{28}$, $–SO_2R^{28}$, wherein R^{28} is a linear, branched or cyclic, one to ten carbon saturated or unsaturated alkyl group;

Y is defined as a moiety selected from the group consisting of: a linear, saturated or unsaturated, one to six carbon alkyl group, optionally substituted with R^{25}, ArR^{25}–, or X; and,

X is defined as a moiety selected from the group consisting of: –OH, $–OR^{25}$, =O, =S, $–O_2CR^{25}$, –SH, $–SR^{25}$, $–SOCR^{25}$, $–NH_2$, $–NHR^{25}$, $–N(R^{25})_2$, $–NHCOR^{25}$, $–NRCOR^{25}$, –I, –Br, –Cl, –F, –CN, $–CO_2H$, $–CO_2R^{25}$, –CHO, $–COR^{25}$, $–CONH_2$, $–CONHR^{25}$, $–CON(R^{25})_2$, –COSH, $–COSR^{25}$, $–NO_2$, $–SO_3H$, $–SOR^{25}$, and $–SO_2R^{25}$;

or a stereoisomer, prodrug or pharmaceutically acceptable salt thereof.

In another embodiment, T–L^1–PT has the following structure (XXV):



XXV

wherein,

R^{26} is selected from the group consisting of H, optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, $-COR^{27}$, $-CSR^{27}$, $-OR^{27}$, $-SR^{27}$, and $-NHR^{27}$, wherein each R^{27} is, independently, of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, or optionally substituted heteroaryl;

R^{29} is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl;

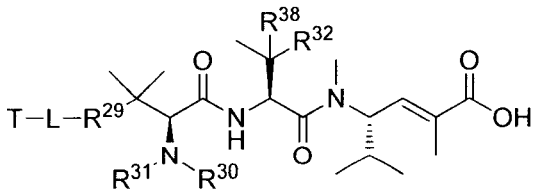
R^{30} is selected from the group consisting of H and C_{1-6} alkyl;

R^{31} is selected from the group consisting of H and C_{1-6} alkyl;

R^{32} and R^{38} are independently selected from the group consisting of H, C_{1-6} alkyl and –SH, with the proviso that both the R^{32} and R^{38} substituents cannot be H;

or a stereoisomer, prodrug or pharmaceutically acceptable salt thereof.

In another embodiment, T–L^1–PT has the following structure (XXVI):



XXVI

wherein,

R^{29} is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl;

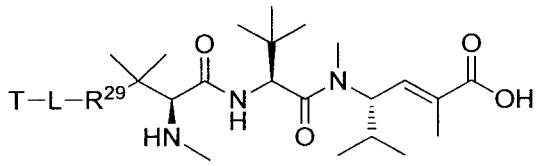
R^{30} is selected from the group consisting of H and C_{1-6} alkyl;

R^{31} is selected from the group consisting of H and C_{1-6} alkyl;

R^{32} and R^{38} are independently selected from the group consisting of H, C_{1-6} alkyl and –SH, with the proviso that both the R^{32} and R^{38} substituents cannot be H;

or a stereoisomer, prodrug or pharmaceutically acceptable salt thereof.

In another embodiment, T–L^1–PT has the following structure (XXVII):



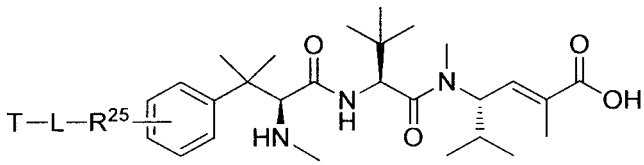
XVII

wherein,

R^{29} is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl;

or a stereoisomer, prodrug or pharmaceutically acceptable salt thereof.

In another embodiment, T–L^1–PT has the following structure (XXVIII):



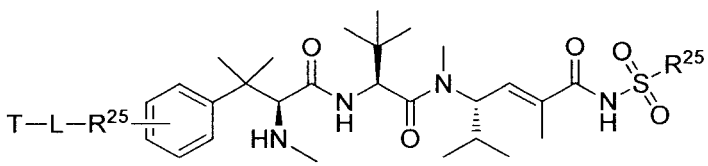
XXVIII

wherein,

R^{25} is defined as a saturated or unsaturated moiety having a linear, branched, or non-aromatic cyclic skeleton containing one to ten carbon atoms, zero to four nitrogen atoms, zero to four oxygen atoms, and zero to four sulfur atoms, and the carbon atoms are optionally substituted with: =O, =S, OH, $-OR^{28}$, $-O_2CR^{28}$, –SH, $-SR^{28}$, $-SOCR^{28}$, $-NH_2$, $-NHR^{28}$, $-N(R^{28})_2$, $-NHCOR^{28}$, $-NR^{28}COR^{28}$, –I, –Br, –Cl, –F, –CN, $-CO_2H$, $-CO_2R^{28}$, –CHO, $-COR^{28}$, $-CONH_2$, $-CONHR^{28}$, $-CON(R^{28})_2$, –COSH, $-COSR^{28}$, $-NO_2$, $-SO_3H$, $-SOR^{28}$, $-SO_2R^{28}$, wherein R^{28} is a linear, branched or cyclic, one to ten carbon saturated or unsaturated alkyl group,

or a stereoisomer, prodrug or pharmaceutically acceptable salt thereof.

In another embodiment, T–L^1–PT has the following structure (XXIX):



XXIX

wherein,

R^{25} is defined as a saturated or unsaturated moiety having a linear, branched, or non-aromatic cyclic skeleton containing one to ten carbon atoms, zero to four nitrogen atoms, zero to four oxygen atoms, and zero to four sulfur atoms, and the carbon atoms are optionally substituted with: =O, =S,

OH, $-OR^{28}$, $-O_2CR^{28}$, –SH, $-SR^{28}$, $-SOCR^{28}$, $-NH_2$, $-NHR^{28}$, $-N(R^{28})_2$, $-NHCOR^{28}$, $-NR^{28}COR^{28}$, –I, –Br, –Cl, –F, –CN, $-CO_2H$, $-CO_2R^{28}$, –CHO, $-COR^{28}$, $-CONH_2$, $-CONHR^{28}$, $-CON(R^{28})_2$, –COSH, $-COSR^{28}$, $-NO_2$, $-SO_3H$, $-SOR^{28}$, $-SO_2R^{28}$, wherein R^{28} is a linear, branched or cyclic, one to ten carbon saturated or unsaturated alkyl group, or a stereoisomer, prodrug or pharmaceutically acceptable salt thereof.

In a further embodiment of the invention, (PT) is a hemiasterlin analog, such as those disclosed in US 7,579,323, which is hereby incorporated by reference in its entirety for all purposes. In a further embodiment of the invention, (PT) is a hemiasterlin analog, such as those disclosed in US Appl. No.: 10/666,722 or US Appl. No.: 10/911,300, each of which is hereby incorporated by reference in its entirety for all purposes.

LINKER MOIETY L^2

Provided are compounds of Formula VII:

$$T\text{–}L^2\text{–}P^2$$

VII

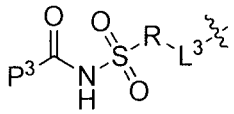
wherein:

T is a targeting moiety comprising a VAR2CSA polypeptide;

L^2 is a linker, or L^2 is absent;

P^2 is a biologically active compound; and

L^2–P^2 has the following structure (III):



III

wherein:

R is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, $-COR^{27}$, $-CSR^{27}$, $-OR^{27}$, and $-NHR^{27}$, wherein each R^{27} is, independently, optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, or R is absent;

P^3 is the remaining portion of compound P^2; and

L^3 is optionally the remaining portion of linker L^2 when L^2 is present.

In a preferred embodiment, R is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl, or R is absent.

Also provided are compounds comprising a payload compound linked to a targeting moiety comprising a VAR2CSA polypeptide in a conjugate that is enzymatically cleavable and capable of releasing payload compound from targeting moiety upon enzymatic cleavage. In some embodiments, the payload compound is a biologically active compound. In some embodiments, the payload compound is a cytotoxic or cytostatic drug.

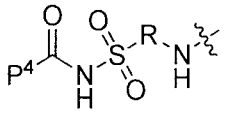
As disclosed herein, *N*-acyl sulfonamide-containing cleavable conjugates may be synthesized such that an *N*-acyl sulfonamide moiety is covalently linked to a chemical group, (R), which is covalently bonded to a nitrogen atom that forms an enzymatically cleavable peptide bond (the junction peptide bond (JPB)) with the carbonyl group of an amino acid that forms part of the amino acid sequence facilitating enzymatic cleavage of the JPB. Moieties similar to N-acyl sulfonamides, such as N-acyl sulfamamides, may also be used.

Accordingly, in one embodiment, the invention provides compounds of Formula VII:

$$T\text{–}L^2\text{–}P^2$$

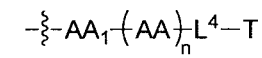
VII

wherein P^2 is a biologically active compound, L^2 is a linker, and T is a targeting moiety comprising VAR2CSA, wherein P^2 has the following structure (V):



V

and wherein L^2–T has the following structure (VI):



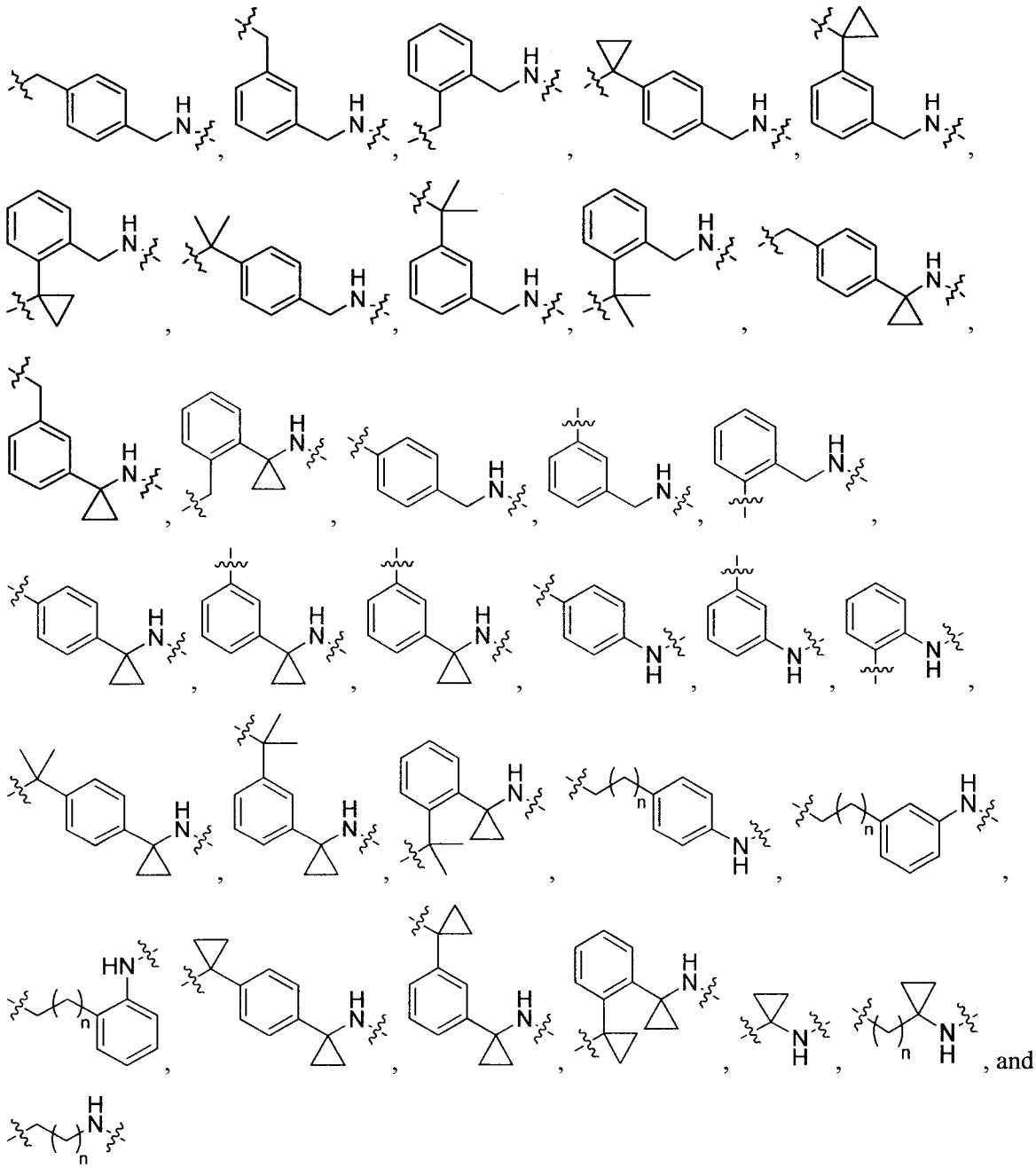
VI

wherein P^4 is the remaining portion of payload compound P^2, wherein the –NH– group bonded to R in Formula V forms a peptide bond (JPB) with AA_1 in formula VI, wherein said JPB is enzymatically cleavable, wherein R is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl, wherein each AA is independently an amino acid, wherein n is an integer from 0 to 25, wherein L^4 is optionally the remaining portion of linker L^2, wherein T is said targeting moiety, and wherein AA_1–$(AA)_n$, taken together comprises an amino acid sequence capable of facilitating enyzmatic cleavage of said JPB.

In some embodiments, R is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl,

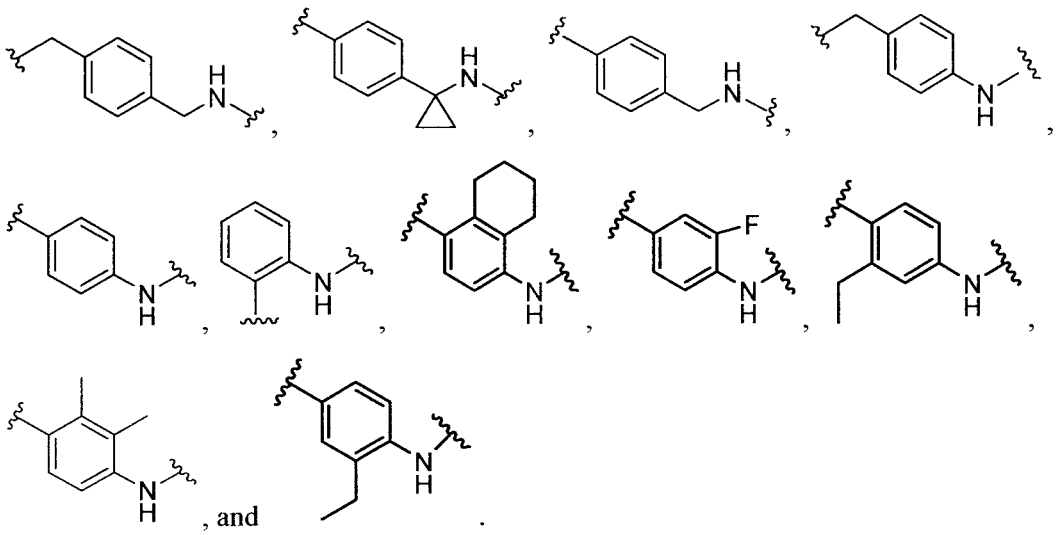
optionally substituted heterocyclyl, optionally substituted heteroaryl, $-COR^{27}$, $-CSR^{27}$, $-OR^{27}$, and $-NHR^{27}$, wherein each R^{27} is, independently, optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, and optionally substituted heteroaryl.

In some embodiments, R is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, and optionally substituted heteroaryl. In some embodiments, –R–NH– of Formula V is selected from:

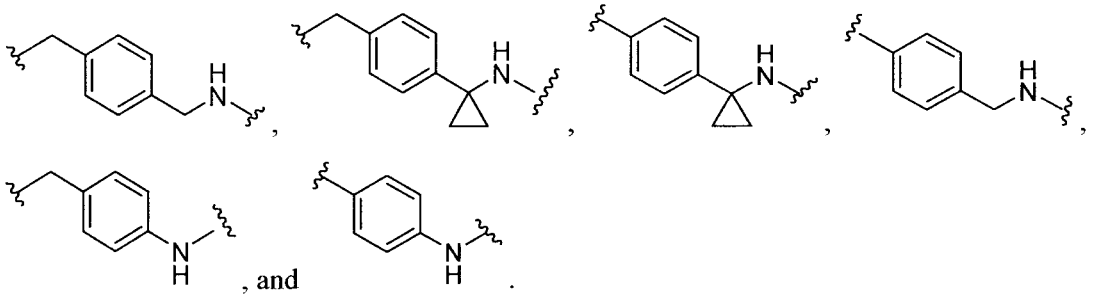


wherein each n is independently an integer from 0-10.

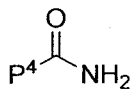
In some embodiments, –R–NH– of Formula V is selected from:



In some embodiments, –R–NH– of Formula V is selected from:



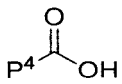
In one embodiment, cleavage of the JPB results in a compound of Formula VIII:



VIII

wherein P^4 corresponds to P^4 in Formula V.

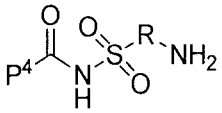
In one embodiment, cleavage of the JPB results in a compound of Formula XXIX:



XXIX

wherein P^4 corresponds to P^4 in Formula V.

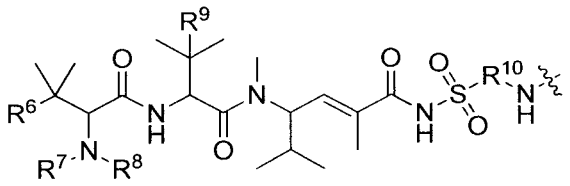
In one embodiment, cleavage of the JPB results in a compound of Formula IX:



IX

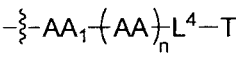
wherein P^4 corresponds to P^4 in Formula V.

In some embodiment, P^2 has the following structure (X):



X

or a stereoisomer, prodrug or pharmaceutically acceptable salt thereof, and L^2–T has the following structure (IV):



VI

wherein:

R^6 is selected from: aryl, C_3-C_7 cycloalkyl, and heteroaryl, each of which is optionally substituted with one or more substituents selected from: C_1-C_4 acylthio, C_2-C_4 alkenyl, C_1-C_4 alkyl, C_1-C_4 alkylamino, C_1-C_4 alkoxy, amino, amino-C_1-C_4 alkyl, halo, C_1-C_4 haloalkyl, hydroxyl, hydroxy-C_1-C_4 alkyl, and thio, wherein C_2-C_4 alkenyl, C_1-C_4 alkylamino and C_1-C_4 alkoxy are further optionally substituted with one substituent selected from C_1-C_4 alkylaryl, hydroxyl, and thio;

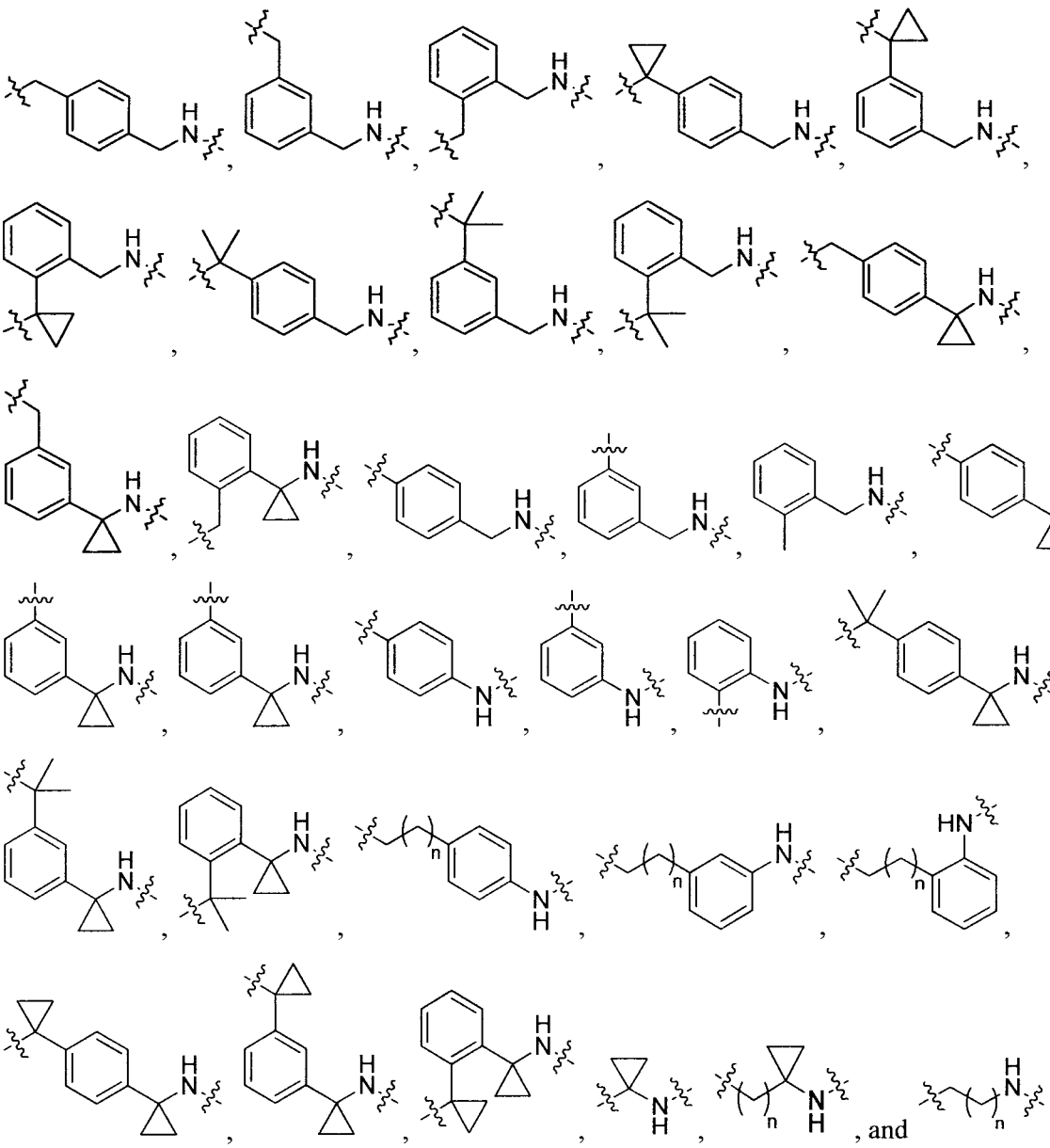
R^7 and R^8 are each independently selected from: H and C_1-C_6 alkyl;

R^9 is selected from the group consisting of C_1-C_6 alkyl and thio; and

wherein the –NH– group bonded to R^{10} in Formula X forms the junction peptide bond (JPB) with AA_1 in Formula IV, wherein the JPB is enzymatically cleavable, wherein each AA is independently an amino acid, wherein n is an integer from 0 to 25, wherein L^3 is the remaining portion (if any) of linker L^2, wherein T is the targeting moiety, AA_1–$(AA)_n$, taken together comprises an amino acid sequence capable of facilitating enyzmatic cleavage of said JPB.

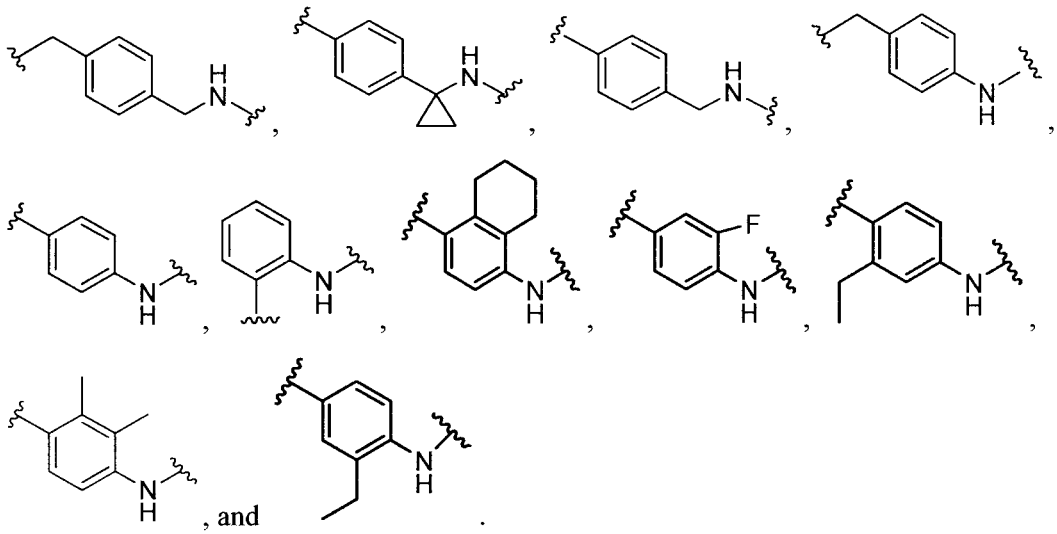
In some embodiments, R^{10} is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted heteroaryl, $–COR^{27}$, $–CSR^{27}$, $–OR^{27}$, and $–NHR^{27}$, wherein each R^{27} is, independently, optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, and optionally substituted heteroaryl.

In some embodiments, R^{10} is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl, and optionally substituted heteroaryl. In some embodiments, $–R^{10}–NH–$ of Formula X is selected from:

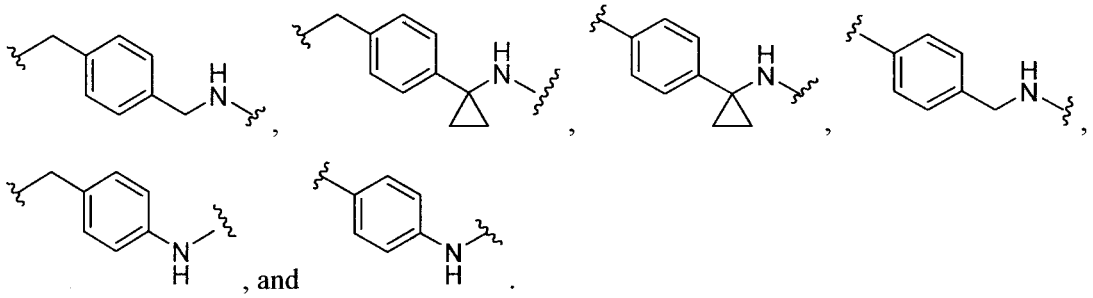


wherein each n is independently an integer from 0-10.

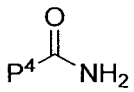
In some embodiments, $-R^{10}-NH-$ of Formula X is selected from:



In some embodiments, $-R^{10}-NH-$ of Formula X is selected from:



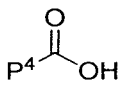
In one embodiment, cleavage of the JPB results in a compound of Formula VIII:



VIII

wherein P^4 corresponds to P^4 in Formula V.

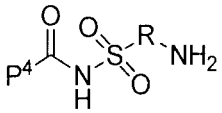
In one embodiment, cleavage of the JPB results in a compound of Formula XXIX:



XXIX

wherein P^4 corresponds to P^4 in Formula V.

In one embodiment, cleavage of the JPB results in a compound of Formula IX:



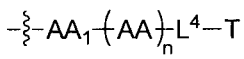
IX

wherein P^4 corresponds to P^4 in Formula V.

The linker moiety L^2 is characterized from the perspective of an assembled conjugate described herein. Accordingly, L^2 as characterized herein does not necessarily but may correspond to

a particular reactant used in the synthesis of a conjugate. The components of L^2 may be contributed by a number of reactants. Accordingly, L^2 is a bifunctional unit that links a payload compound (P^2) to a targeting moiety (T) to form a conjugate compound, T–L^2–P^2, that may be cleaved enzymatically at the junction peptide bond (JPB) between P^2 and L^2 to release P^2. Such conjugates allow the selective delivery of payload P^2 to target cells (*e.g.*, tumor cells).

The linker L^2 and the targeting moiety T taken together have the following structure (VI):



VI

wherein the carbonyl of AA_1 forms a peptide bond referred to herein as the junction peptide bond (JPB) with the –NH– group bonded to R in Formula V, wherein the JPB is enzymatically cleavable, wherein each AA is independently an amino acid, wherein x is an integer from 0 to 25, wherein L^3 is the remaining portion (if any) of linker L^2, wherein T is the targeting moiety, and wherein AA_1–$(AA)_n$ comprises an amino acid sequence capable of facilitating enyzmatic cleavage of the JPB.

The amino acid unit AA_1–$(AA)_n$, comprises a recognition sequence that provides for cleavage of the junction peptide bond (JPB) to release payload P^2 from the targeting moiety T. Any sequence capable of providing for such enzymatic cleavage may be used. Such sequences include, but are not limited to, applicable sequences described in US 6,214,345. For example, amino acid sequences known in the art to direct cleavage of a peptide bond linking a PABC self-immolative unit directly to the amino acid sequence may be used in the present invention. Additional amino acid sequences useful in the present invention can be readily determined experimentally by the artisan of reasonable skill. In certain embodiments, an amino acid unit, AA_1–$(AA)_n$ allows for cleavage of the (JPB) by a protease, thereby facilitating release of payload P^2 from the conjugate upon exposure to such proteases. In certain embodiments, these include intracellular proteases, such as lysosomal enzymes. In yet further embodiments, these include extracellular proteases.

Exemplary amino acid units $(AA)^1$-$(AA)_x$ include, but are not limited to, a dipeptide, a tripeptide, a tetrapeptide, and/or a pentapeptide. Exemplary dipeptides include: Val-Cit, Ala-Phe, Phe-Lys, Val-Ala, Val-Lys(Ac), Phe-Lys(Ac), or Me-Val-Cit. It is noted that while the naming convention for peptides and proteins is to list amino acid sequence from N-terminus to C-terminus, the configuration of the JPB is such that $(AA)^1$ is the C-terminus amino acid in the $(AA)^1$-$(AA)_x$ amino acid sequence. Accordingly, in an embodiment where the amino acid sequence facilitating enzymatic cleavage of the JPB was valine-citrulline, $(AA)^1$ in formula (III) would be citrulline and the carbonyl group of citrulline would form JPB with the -NH- group bonded to (R) in structure (II). In some embodiments, additional amino acids are linked to valine-citrulline through the N-terminus of valine and, accordingly, "x" for $(AA)_x$ is an integer greater than one.

Exemplary tripeptides include: Gly-Val-Cit, Pro-Pro-Pro, D-Ala-Phe-Lys, (D)-Val-Leu-Lys, Gly-Gly-Arg, and Ala-Ala-Asn. For illustration and clarity, when the tripeptide is (gly-val-cit), $(AA)^1$ of formula (III) is citrulline. An amino acid unit may comprise amino acid residues that occur naturally, as well as minor amino acids and non-naturally occurring amino acid analogs, such as citrulline. D-amino acids are included for use in the invention. Amino acid units can be designed and optimized in their selectivity for enzymatic cleavage by a particular enzyme, for example, a tumor-associated protease, cathepsin B, C and D, or a plasmin protease.

Exemplary tetrapeptides include: Lys-Ser-Gly-Arg, Gly-Phe-Leu-Gly, Leu-Ser-Gly-Arg, Ala-Leu-Ala-Leu, Gly-Gly-Gly-Arg-Arg, Gly-Lys-Ala-Phe-Arg-Arg, and HomoGly-Arg-Ser-Arg-Gly

Exemplary amino acid sequences for use in linkers of the invention include the amino acid sequences within Phe-Lys, Val-Lys, Ala-Lys, Val-Cit, Phe-Cit, Leu-Cit, Ile-Cit, Trp-Cit, Phe-Arg. These sequences have been used for release of doxorubicin. See, for example, Table 1, Dubowchik, Firestone *et al. Bioconjugate Chem.* **2002**, *13*, 855-869 and references contained therein. Another exemplary amino acid sequence for use in linkers of the present invention is Pro-Pro (see, for example, Gianolio *et al. Cancer Chemother Pharmacol* **2012** *70*, 439–449). See also Firestone et al., US 6,214,345 for amino acid sequences useful in the present invention. See also Miao et al., WO 2013/173392 for amino acid sequences useful in the present invention, including but not limited to amino acid sequences comprising non-natural amino acids. See also Dubowchik et al., Bioorganic & Med. Chem. Letters 8:3341-3346, 1998. See also Burke et al., Bioorganic & Med. Chem. Letters 19:2650-2653, 2009. See also Jeffrey et al., Bioorganic & Med. Chem. Letters 16:358-362, 2006. The artisan of reasonable skill will appreciate that additional amino acids may be included in the linker (L) to the N-terminus side of the amino acid sequence that is facilitating enzymatic cleavage of the JPB.

In one example, the JPB is cleavable by a protease that is associated with a disease. In another example, the JPB is cleavable by a protease that is up-regulated or associated with cancers in general. In still another example, the JPB is cleavable by a protease secreted by cancer-associated cells.

In another example, the JPB is cleavable by an enzyme that is up-regulated or associated with a specific cancer.

In certain embodiments, the remaining portion of linker (L^3) includes a stretcher moiety (S) between the amino acid unit, AA_1–$(AA)_n$ and the targeting moiety as shown in the following structures (XIa) and (XIb):

$$-AA_1-(AA)_n-S-L^4-T$$

XIa

$$-AA_1-(AA)_n-L^4-S-T$$

XIb

wherein the carbonyl of AA_1 forms a peptide bond referred to herein as the junction peptide bond (JPB) with the –NH– group bonded to R in Formula V, wherein the JPB is enzymatically cleavable, wherein each AA is independently an amino acid, wherein n is an integer from 0 to 25, wherein L^4 is the remaining portion (if any) of L^3, wherein S is the stretcher unit, wherein T is the targeting moiety, and wherein AA_1–$(AA)_n$ comprises an amino acid sequence capable of facilitating enyzmatic cleavage of the JPB. In particular embodiments, this stretcher is as described in US 7,964,566 and US 6,214,345.

PAYLOAD MOIETY (P^2)

Provided are compounds of Formula VII:

$$T–L^2–P^2$$

VII

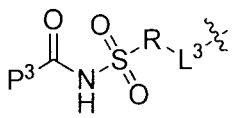
wherein:

T is a targeting moiety comprising a VAR2CSA polypeptide;

L^2 is a linker, or L^2 is absent;

P^2 is a biologically active compound; and

L^2–P^2 has the following structure (III):



III

wherein:

R is selected from the group consisting of optionally substituted alkyl, optionally substituted alkylamino, optionally substituted cycloalkyl, optionally substituted aryl, optionally substituted heterocyclyl and optionally substituted heteroaryl, or R is absent;

P^3 is the remaining portion of compound P^2; and

L^3 is optionally the remaining portion of linker L^2 when L^2 is present.

As with the linker moiety L^2, the payload P^2 is characterized from the perspective of an assembled conjugate described herein. Accordingly, the payload P^2 as characterized herein does not necessarily but may correspond to a particular reactant used in the synthesis of a conjugate. The components of the payload P^2 may be contributed by a number of reactants. Included within the scope of P^2 are precursors of biologically active compounds that may be converted to biologically active compounds *in vivo*.

A wide variety of compounds may be used to assemble desirable payload components of a conjugate described herein. Any compound that is functional as an amide as in Formula VIII or as a

compound containing an *N*-acyl sulfonamide-R-NH_2 group as in Formula IX could be delivered to a target cell or tissue using the present conjugate technology. Any precursor compounds that can be used (directly, or following appropriate modification) to produce amides of Formula VIII or *N*-acyl sulfonamide-R-NH_2 compounds of Formula IX find use in the invention. Particularly preferred are amide containing drugs, carboxylic acid containing drugs that have active amide derivatives, carboxylic acid containing drugs, and drugs having the Formula IX. The route of synthesis and the particular reactants used to produce compounds of Formula VII are not limiting. It will be appreciated that in combination with the group "R", compounds of formula IX may be similar to *N*-acyl sulfonamides (*e.g.*, sulfamamides).

In some embodiments, compounds of Formula VII can be used to deliver biologically active compounds of Formula VIII or IX. Suitable payload compounds P^2 that may be advantageously delivered by way of compositions described herein to targeted locations include, *e.g.*, anti-inflammatory agents, cytotoxic agents, and anti-cancer drugs. In some embodiments, compounds of Formula VIII and IX show cytotoxic or cytostatic activity.

Non-limiting examples of cytotoxic payloads which may be fused or conjugated to targeting moieties comprising VAR2CSA polypeptides described herein, are chemotherapeutics selected from calicheamycin, cisplatin, adriamycin, auristatin, doxorubicin, maytansinoid, taxol, ecteinascidin, geldanamycin, methotrexate and their derivatives, and combinations thereof and the like suitable for cancer therapy. Examples of cytotoxic proteins fused to targeting moieties comprising VAR2CSA polypeptides are Pseudomonas exotoxin A, diphtheria toxin, ricin toxin, pokeweed antiviral protein, saporin, gelonin and variants hereof.

The payload molecule is preferably selectively guided to a cell, which expresses plCSA and includes anticancer agents, radioisotopes, toxins, cytostatic or cytolytic drugs, *etc.* Anticancer agents comprise, for example, anthracyclins (doxorubicin, daunorubicin, epirubicin, idarubicin, valrubicin, mitoxantrone), platinium and non-platinium based alkylating agents (cisplatin, carboplatin, oxaliplatin, mechlorethamine, cyclophosphamide, chlorambucil, ifosfamide, busulfan, carmustine, dacarbazine, lomustine, procarbazine), vinca alkaloids (vincristine, vinblastine, vinorelbine, vindesine), taxanes (taxol and decetaxel), topoisomerase I inhibitors (camptothecin, irinotecan, topotecan), topoisomerase II inhibitors (amsacrine, etoposide, etoposide phosphate, teniposide and other alkaloid-derivates naturaly occurring in the root of American Mayapple (*Podophyllum peltatum*)), non-anthracyclin cytotoxic antibiotics (dactinomycin, bleomycin, plicamycin and mitomycins), Anti-steroids (such as aminoglutethimide), nucleoside analogues (cytarabidine, fluorouracil and mercaptopurine), antimetabolites (methotrexate and thioguanine), dichlorodiphenyltrichloroethane analogues (like mitotane), and reactive oxygen species (ROS)-inducing compounds (including but not limited to piperlongumine, and beta-phenylethyl isothiocyanate). Other anticancer agents are described, for example, in Goodman and Gilman, "The

Pharmacological Basis of Therapeutics", 8th Edition, 1990, McGraw-Hill, Inc., in particular Chapter 52 (Antineoplastic Agents (Paul Calabresi and Bruce A. Chabner). Toxins may be proteins such as pokeweed antiviral protein, cholera toxin, pertussis toxin, ricin, gelonin, abrin, diphtheria exotoxin or Pseudomonas exotoxin. Toxin residues may also be high energy-emitting radionuclides such as cobalt-60. A VAR2CSA polypeptide conjugate may be used together with cell-penetrating peptides (CPP) to facilitate transport of the conjugate across cell plasma membranes. Cell-penetrating peptides have found numerous applications in medicine as drug delivery agents in the treatment of different diseases including cancer and virus inhibitors. Examples on CPP include but are not limited to: trans-activating transcriptional activator (Tat) from human immunodeficiency virus; pep-1 (ChariotTM); R8, azo-R8; SMoC. (Okuyama M *et al.* Nat Methods. 2007 Feb;4(2):153-9M; Soane L and Fiskum GJ Neurochem. 2005 Oct;95(1):230-43; Loudet A *et al.* Org Biomol Chem. 2008 Dec 21;6(24):4516-22).

In some embodiments, a targeting moiety comprising a VAR2CSA polypeptide is conjugated with an anti-inflammatory agent, including steroid hormones. Cartilage and scar tissue is known to contain CSPG in high amounts. Accordingly, it is useful to direct anti-inflammatory agents such as non-steroid anti-inflammatory compounds, disease modifying anti-rheumatic drugs (such as methotrexate, azathioprine, sulfasalazine, ciclosporine, pennicillamine, leflunomide, or gold), biological anti-rheumatic drugs (such as tumor necrosis factor inhibitors, interleukin-1-receptor antagonists, CD20-antibody, insulin growth factor 1) and steroid hormones or alternative compounds to such tissues.

In some embodiments, P^2 is a cytotoxic compound.

In some embodiments, P^2 is a cytotoxic compound, for example, a compound disclosed in U.S. 7,579,323; WO 2004/026293; U.S. 8,129,407; US 2014/0227295; WO 2013/068874; US 2013/0095123; US 2013/0190243; WO 2014/126198; EP 2740493; WO 2014086942; WO 2013072813; WO 2012166559; WO 2012166560; WO 2012123423; WO 2011154359; WO 2006063707; WO 2003008378; WO 2002000263; US 2013/224,228; WO 2013/085925; WO 2014/009774; US 8,476,451; U.S. 2011/0027274; or related applications or patents, or Lundquist *et al.*, Organic Letters, (3), pp. 781-783, 2001; Domling *et al.*, Angew. Chem. Int. Ed. 2006, 45, 7235 – 7239; Kaur *et al.*, Biochem J., (2006), 396:235-242; Steinmetz *et al.*, Angew. Chem. Int. Ed. 2004, 43, 4888 –4892; Khalil *et al.*, ChemBioChem 2006, 7, 678 – 683; Peltier *et al.*, J. Am. Chem. Soc. 2006, 128, 16018-16019.

In some embodiments, P^2 is a microtubule disrupting peptide toxin.

In some embodiments, P^2 is hemiasterlin or an analog thereof.

In some embodiments, P^2 is tubulysin or an analog thereof.

In some embodiments, P^2 is auristatin or an analog thereof.

In some embodiments, the cytotoxic compound is selected from: calicheamycin, auristatin, doxorubicin, maytansinoid, taxol, ecteinascidin, geldanamycin, methotrexate Pseudomonas exotoxin A, diphtheria toxin, ricin toxin, pokeweed antiviral protein, saporin, gelonin, pyrrolobenzodiazepines (PBDs) and functional variants, fragments, and combinations thereof.

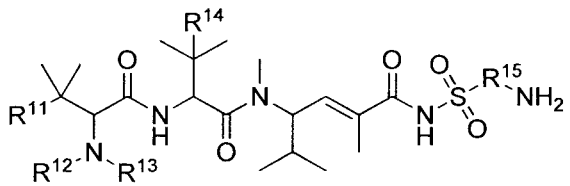
In some embodiments, the cytotoxic compound is a polyketide from *Lithoplocamia lithistoides*. Examples of polyketides from *Lithoplocamia lithistoides* include those disclosed in Martín *et al.*, J. Am. Chem. Soc. 2013, 135, 10164-10171. In some embodiments, the polyketide from *Lithoplocamia lithistoides* is selected from: PM050489 and PM060184.

In some embodiments, cytotoxic compound is a synthetic chemotoxin not derived from a naturally occurring compound.

In some embodiments, P^2 is an anti-inflammatory compound.

In some embodiments, P^2 is a microtubule disrupting peptide toxin. In one embodiment, the microtubule disrupting peptide toxin is hemiasterlin or an analog thereof. In another embodiment, the microtubule disrupting peptide toxin is HTI-286 or an analog thereof. In one embodiment, the microtubule disrupting peptide toxin is auristatin or an analog thereof. In one embodiment, the microtubule disrupting peptide toxin is a compound having structure XIII, XIV, or XIX .

In some embodiments, P^2 is a compound of Formula XIII



XIII

or a stereoisomer, prodrug or pharmaceutically acceptable salt thereof, wherein:

R^{11} is selected from: aryl, C_3-C_7 cycloalkyl, and heteroaryl, each of which is optionally substituted with one or more substituents selected from: C_1-C_4 acylthio, C_2-C_4 alkenyl, C_1-C_4 alkyl, C_1-C_4 alkylamino, C_1-C_4 alkoxy, amino, amino-C_1-C_4 alkyl, halo, C_1-C_4 haloalkyl, hydroxyl, hydroxy-C_1-C_4 alkyl, and thio, wherein C_2-C_4 alkenyl, C_1-C_4 alkylamino and C_1-C_4 alkoxy are further optionally substituted with one substituent selected from C_1-C_4 alkylaryl, hydroxyl, and thio;

R^{12} and R^{13} are each independently selected from: H and C_1-C_6 alkyl;

R^{14} is selected from the group consisting of C_1-C_6 alkyl and thio; and

R^{15} has the same definition as R^{10} in Formula X.

In one embodiment R^{11} is selected from: phenyl, 1*H*-indol-3-yl, 1-methyl-1*H*-indol-3-yl, cyclohexyl, 4-methoxyphenyl, 2-methoxyphenyl, 3,5-dimethylphenyl, and *m*-tolyl.

In another further embodiment, R^{12}, R^{13} and R^{14} are each methyl.

In another further embodiment, R^{12} is H, R^{13} is methyl, and R^{14} is methyl.

METHODS OF PREPARING VAR2CSA-DRUG CONJUGATES

Provided are methods of making compounds of Formula I. As will be appreciated by the artisan of reasonable skill, a wide variety of means are available to covalently link T–L–P. Any known method may be used to link the conjugate components. Any known linker technology may be used to link T to P. Further, T, L, and P may be modified in any suitable manner, as recognized by the artisan of reasonable skill, in order to facilitate conjugate formation.

Compounds of Formula I can be produced using a wide range of synthetic routes and a wide range of reactants. For example, the *N*-acyl sulfonamide moiety and the R group of Formula III may be present in the same reactant or different reactants. The *N*-acyl sulfonamide moiety may be present on a single reactant or may be formed by two reactants in a conjugation reaction step. The JPB may be intact within a reactant or may be formed by two reactants in a conjugation reaction step. The JPB may be intact within a single reactant that also contains the amino acid sequence facilitating enzymatic cleavage of the JPB, or the amino acid sequence facilitating enzymatic cleavage may be formed and brought together with the JPB by multiple reactants in a conjugation reaction step.

In some embodiments, compounds of Formula I are prepared by the conjugation of T with a precursor of L–P of Formula XII:

FG–L–P

XII

wherein FG is a functional group that forms a covalent bond with one or more atoms of the targeting moiety . In further embodiments, FG forms a bond with a heteroatom of the targeting moiety. In particular embodiments, the FG group comprises a maleimide. As will be appreciated by the artisan of reasonable skill, additional moieties and bonding technologies may be used, including but not limited to transglutaminase sequences, 2-bromoacetamide chemistry, glycosylation chemistries, and others. See for example the linkage chemistry disclosed in WO2013173391, WO2013173392, WO2013173393, and US 7,964,566.

INDICATIONS

The compounds described herein may be used in a wide range of indications associated with expression, such as inappropriate expression of plCSA, such as in various cancers, such as metastatic cancers including, but not limited to, melanomas, sarcomas, oligodendrocytomas, brain tumors, leukemias, lymphomas, and carcinomas of the lung, breast, urothelium, colon, pancreas, and liver. The compounds described herein may also be used for cancer stem cells and accordingly target the cells before development into a cancer. Other conditions associated with expression, such as inappropriate expression of plCSA are conditions of the cartilage and/or the development of scar tissue.

Accordingly, provided are methods for the treatment of any indication associated with expression, such as inappropriate expression of plCSA, such as in cancer, arthritis, arthrosis, multiple sclerosis, pathological conditions caused by neural damage, conditions of the cartilage and scar tissue, such as in rheumatism, cartilage repair or wound healing, or in psoriasis; the methods comprising administering a therapeutically or prophylactically effective amount of a compound described herein to a subject in need thereof.

Also provided are compounds for the treatment of any indications associated with a condition involving expression, such as inappropriate expression of plCSA, such as in cancer, arthritis, arthrosis, multiple sclerosis, pathological conditions caused by neural damage, conditions of the cartilage and scar tissue, such as in rheumatism, cartilage repair or wound healing, or in psoriasis.

The compounds described herein may be used in identifying, tracking and targeting distant micro-metastasis *in vivo*. Virtually all primary tumors, including cancers of the hematopoietic system, have the potential of developing into metastatic disease, which is highly associated with poor therapeutic outcome of the patients.

In some embodiments, the compounds described herein are useful in the treatment of malignant melanoma cancer including cutaneous, ocular and conjuctival melanoma having CSPG4 with plCSA chains on the surface of the melanoma cells. This GAG chain is believed to be involved in mitoses and metastases. However, CSPG4 is not only specific to melanoma. Micro- and tissue array analyses, performed on data from large panels of human tissue and cell lines, suggest that CSPG4 and other types of plCSA-containing proteoglycans may be present on a wide range of cancer types originating from all three cellular germ layers. These cancer types include carcinomas (breast carcinoma, pancreatic carcinoma, ovarian carcinoma, endometrial carcinoma, hepatocellular carcinoma, lung carcinoma, colon carcinoma, prostate carcinoma, cervix carcinoma, testis carcinoma, basal cell skin carcinoma, clear cell renal cell carcinoma, kreatinized head and neck squamous cell carcinoma, skin squamous cell carcinoma, vulvar kreatinized squamous cell carcinoma and vulvar basal cell carcinoma), sarcomas (breast liposarcoma, fibrosarcoma, dedifferentiated chondro- and liposarcoma, leiomyosarcoma, liposarcoma, myxoid liposarcoma, uterine corpus leiomyosarcoma, osteosarcoma, Ewing sarcoma and rhabdomyosarcoma), hematopoietic cancers (chronic lymphatic leukemia (CLL), acute lymphatic leukemia (ALL), acute myeloid leukemia (AML), B-cell, T-cell and large granular lymphoma), tumors of neuroepithelial tissue, such as astrocytomas (pleomorphic xanthoastrocytoma, fibrillary astrocytomas, anaplastic astrocytoma, glioblastoma multiforme), oligodrendroglioma, ependymoma, choroid plexus tumor, oligoastrocytoma, gliosarcoma, ganglioglioma, retinoblastoma, neurocytoma, neuroblastomas (esthesioneuroblastoma and ganglioneuroblastoma), medulloblastoma, atypical teratoid rhabdoid tumors and all types of neuroendocrine cancer.

Chondroitin sulfate proteoglycans (CSPG) also constitute an important component of the extracellular matrix of the central nervous system (CNS) including the eye, and of joint cartilage. Extra-cellular CSPG is critically involved in the pathogenesis of arthritis and the lack of regeneration after neural damage. Loss of extra-cellular CSPG is critical for the development of arthritis and arthrosis, and high local concentrations of extra-cellular CSPG prevents neural out-growth after neural damage. Accordingly, the compounds described herein may be used not only in the treatment of indications associated with malignant growth, such as in cancers, but also to either increase or decrease CSPG presence in the extracellular environment in order to treat arthritis, athrosis and to enhance neural recovery after neurite damage, including multiple sclerosis.

The compounds described herein may be used to target compounds that prevent degradation of or repair extracellular CSPG such as growth hormones, anti-inflammatory compounds or protein inhibitors, to cartilage tissue, joints, and neural tissue.

The compounds described herein may be used to target compounds that enhance degradation or prevent production of extracellular CSPG such as chondroitinase ABC, which cut the sugar chains of the protein core of CSPG molecules. Xylocides, which reduce CSPG production, or drugs that inhibit enzymes important for CSPG production such as chondroitin synthase or chondroitin polymerizing factor (such as 4-fluoro-glucosamine, p-nitrophenyl-beta-D-xyloxide, 4-methyl-umbelliferyl-beta-D-xylopyranoside), to damaged neural tissue.

In some embodiments, the cancer is selected from cutaneous, ocular or conjuctival melanoma; carcinomas (triple negative- and metaplastic breast carcinoma, pancreatic carcinoma, ovarian carcinoma, endometrial carcinoma, hepatocellular carcinoma, lung carcinoma, colon carcinoma, prostate carcinoma, cervix carcinoma, testis carcinoma, basal cell skin carcinoma, clear cell renal cell carcinoma, kreatinized head and neck squamous cell carcinoma, skin squamous cell carcinoma, vulvar kreatinized squamous cell carcinoma and vulvar basal cell carcinoma); sarcomas (breast liposarcoma, fibrosarcoma, dedifferentiated chondro- and liposarcoma, leiomyosarcoma, liposarcoma, myxoid liposarcoma, uterine corpus leiomyosarcoma, osteosarcoma, Ewing sarcoma and rhabdomyosarcoma); hematopoietic cancers (chronic lymphatic leukemia (CLL), acute lymphatic leukemia (ALL), acute myeloid leukemia (AML), B-cell, T-cell and large granular lymphoma); tumors of neuroepithelial tissue, such as astrocytomas (pleomorphic xanthoastrocytoma, fibrillary astrocytomas, anaplastic astrocytoma, glioblastoma multiforme), oligodrendroglioma, ependymoma, choroid plexus tumor, oligoastrocytoma, gliosarcoma, ganglioglioma, retinoblastoma, neurocytoma, neuroblastomas (esthesioneuroblastoma and ganglioneuroblastoma), medulloblastoma, and atypical teratoid rhabdoid tumors; and any other plCSA-expressing cancer subtype. In some embodiments, the cancer is selected from all plCSA-expressing malignancies including carcinomas (including but not limited to breast carcinoma, pancreatic carcinoma, ovarian carcinoma, endometrial carcinoma, hepatocellular carcinoma, lung carcinoma, colon carcinoma, prostate carcinoma, cervix carcinoma, testis carcinoma,

basal cell skin carcinoma, clear cell renal cell carcinoma, head and neck squamous cell carcinoma, skin squamous cell carcinoma, vulvar kreatinized squamous cell carcinoma and vulvar basal cell carcinoma); sarcomas (including but not limited to fibrosarcoma, dedifferentiated chondro- and liposarcoma, leiomyosarcoma, liposarcoma, myxoid liposarcoma, uterine corpus leiomyosarcoma, osteosarcoma, Ewing sarcoma and rhabdomyosarcoma, synovial sarcoma, solitary fibrous tumor), hematopoietic cancers (including but not limited to chronic lymphatic leukemia (CLL), acute lymphatic leukemia (ALL), acute myeloid leukemia (AML), b-cell, t-cell and large granular lymphoma); tumors of neuroepithelial tissue, such but not limited to astrocytomas (pleomorphic xanthoastrocytoma, fibrillary astrocytomas, anaplastic astrocytoma, glioblastoma multiforme), oligodrendroglioma, ependymoma, choroid plexus tumor, oligoastrocytoma, gliosarcoma, ganglioglioma, retinoblastoma, neurocytoma, neuroblastomas (esthesioneuroblastoma and ganglioneuroblastoma), medulloblastoma, atypical teratoid rhabdoid tumors; and all types of neuroendocrine cancer.

Solid tumors contemplated for treatment using the presently disclosed compounds include but are not limited to: sarcoma, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon cancer, colorectal cancer, kidney cancer, pancreatic cancer, bone cancer, breast cancer, ovarian cancer, prostate cancer, esophageal cancer, stomach cancer (*e.g.*, gastrointestinal cancer), oral cancer, nasal cancer, throat cancer, squamous cell carcinoma (*e.g.*, of the lung), basal cell carcinoma, adenocarcinoma (*e.g.*, of the lung), sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, uterine cancer, testicular cancer, small cell lung carcinoma, bladder carcinoma, lung cancer, non-small cell lung cancer, epithelial carcinoma, glioma, glioblastoma, multiforme astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, skin cancer, melanoma, neuroblastoma, and retinoblastoma. Blood-borne cancers contemplated for treatment using the presently disclosed compounds include but are not limited to: acute lymphoblastic leukemia "ALL", acute lymphoblastic B-cell leukemia, acute lymphoblastic T-cell leukemia, acute myeloblastic leukemia "AML", acute promyelocytic leukemia "APL", acute monoblastic leukemia, acute erythroleukemic leukemia, acute megakaryoblastic leukemia, acute myelomonocytic leukemia, acute nonlymphocyctic leukemia, acute undifferentiated leukemia, chronic myelocytic leukemia "CML", chronic lymphocytic leukemia "CLL", hairy cell leukemia, and multiple myeloma. Acute and chronic leukemias contemplated for treatment using the presently disclosed compounds include but are not limited to: lymphoblastic, myelogenous,

lymphocytic, and myelocytic leukemias. Lymphomas contemplated for treatment using the presently disclosed compounds include but are not limited to: Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and polycythemia vera. Other cancers contemplated for treatment using the presently disclosed compounds include but are not limited to: peritoneal cancer, hepatocellular cancer, hepatoma, salivary cancer, vulval cancer, thyroid, penile cancer, anal cancer, head and neck cancer, renal cell carcinoma, acute anaplastic large cell carcinoma, and cutaneous anaplastic large cell carcinoma.

Non-limiting examples of disorders to be treated herein include benign and malignant tumors; leukemia and lymphoid malignancies, in particular breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic, prostate or bladder cancer; neuronal, glial, astrocytal, hypothalamic and other glandular, macrophagal, epithelial, stromal and blastocoelic disorders, autoimmune disease, inflammatory disease, fibrosis, and infectious disease. Given the characteristics, and particularly the potency of the subject compounds, it will be apparent to the artisan of reasonable skill that the compounds described herein may be indicated for use to treat any disease where exertion of a cytotoxic or cytotoxic effect on a target cell is desirable. Cancers, including, but not limited to, a tumor, metastasis, or other disease or disorder characterized by uncontrolled or undesired cell growth, can be treated or prevented by administration of the presently disclosed compounds.

A therapeutically effective amount of compound in respect of cancer treatment may reduce the number of cancer cells; reduce the tumor size; inhibit (*i.e.*, slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; increase survival time; and/or relieve to some extent one or more of the symptoms associated with the cancer. To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. Compounds of the present invention are preferably cytotoxic. For cancer therapy, efficacy can, for example, be measured by assessing the time to disease progression (TTP) and/or determining the response rate (RR).

The compounds described herein can also be used in an *in vitro* or *ex vivo* fashion, such as for the treatment of certain cancers, including, but not limited to leukemias and lymphomas, such treatment involving autologous stem cell transplants. This can involve a multi-step process in which the animal's autologous hematopoietic stem cells are harvested and purged of all cancer cells, the animal's remaining bone-marrow cell population is then eradicated via the administration of a high dose of a compound described herein with or without accompanying high dose radiation therapy, and the stem cell graft is infused back into the animal. Supportive care is then provided while bone marrow function is restored and the animal recovers.

Combination Therapy

In other embodiments, methods for treating or preventing cancer are provided, including administering to a patient in need thereof an effective amount of a compound disclosed herein in combination with an additional method of treatment. In some embodiments, the additional method of treatment includes treatment with a chemotherapeutic agent. In one embodiment the chemotherapeutic agent is that with which treatment of the cancer has not been found to be refractory. In another embodiment, the chemotherapeutic agent is that with which the treatment of cancer has been found to be refractory. The compound described herein may be administered before, after, or at the same time as the chemotherapeutic agent.

Suitable anticancer agents include, but are not limited to, methotrexate, taxol, L-asparaginase, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclophosphamide, ifosfamide, nitrosoureas, cisplatin, carboplatin, mitomycin, dacarbazine, procarbizine, topotecan, nitrogen mustards, cytoxan, etoposide, 5-fluorouracil, BCNU, irinotecan, camptothecins, bleomycin, doxorubicin, idarubicin, daunorubicin, actinomycin D, dactinomycin, plicamycin, mitoxantrone, asparaginase, vinblastine, vincristine, vindesine, vinorelbine, paclitaxel, and docetaxel.

Other examples of chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, treosulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; TLK 286 (TELCYTA™); acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, and uracil mustard; triazines such as decarbazine; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; epipodophyllins, such as etoposide, teniposide, topotecan, 9-aminocamptothecin, camptothecin orcrisnatol; bisphosphonates, such as clodronate; antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gamma1I and calicheamicin omegal1 (see, *e.g.*, Agnew, Chem. Intl. Ed. Engl., 33:183-186 (1994)) and anthracyclines such as annamycin, AD 32, alcarubicin, daunorubicin, dexrazoxane, DX-52-1, epirubicin, GPX-100, idarubicin, KRN5500, menogaril, dynemicin, including dynemicin A, an esperamicin, neocarzinostatin chromophore and

related chromoprotein enediyne antibiotic chromophores, aclacinomysins, actinomycin, authramycin, azaserine, bleomycins (*e.g.*, A2 and B2), cactinomycin, carabicin, caminomycin, carzinophilin, chromomycinis, dactinomycin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, liposomal doxorubicin, and deoxydoxorubicin), esorubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin; photodynamic therapies, such as vertoporfin (BPD-MA), phthalocyanine, photosensitizer Pc4, and demethoxy-hypocrellin A (2BA-2-DMHA); folic acid analogues such as denopterin, pteropterin, and trimetrexate; dpurine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, and thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, cytosine arabinoside, dideoxyuridine, doxifluridine, enocitabine, and floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, and testolactone; anti-adrenals such as aminoglutethimide, mitotane, and trilostane; folic acid replenisher such as folinic acid (leucovorin); aceglatone; anti-folate anti-neoplastic agents such as ALIMTA®, LY231514 pemetrexed, dihydrofolate reductase inhibitors such as methotrexate and trimetrexate; anti-metabolites such as 5-fluorouracil (5-FU) and its prodrugs such as UFT, S-1 and capecitabine, floxuridine, doxifluridine and ratitrexed; and thymidylate synthase inhibitors and glycinamide ribonucleotide formyltransferase inhibitors such as raltitrexed (TOMUDEX®, TDX); inhibitors of dihydropyrimidine dehydrogenase such as eniluracil; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids and taxanes, *e.g.*, TAXOL® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE™ Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, IL), and TAXOTERE® doxetaxel (Rhone-Poulenc Rorer, Antony, France); chloranbucil; gemcitabine (GEMZAR®); 6-thioguanine; mercaptopurine; platinum; platinum analogs or platinum-based analogs such as cisplatin, oxaliplatin and carboplatin; vinblastine (VELBAN®); etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); vinca alkaloid; vinorelbine (NAVELBINE®); velcade; revlimid; thalidomide; IMiD3; lovastatin; verapamil; thapsigargin; 1-

methyl-4-phenylpyridinium; cell cycle inhibitors such as staurosporine; novantrone; edatrexate; daunomycin; mitoxantrone; aminopterin; xeloda; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); vitamin D3 analogs, such as EB 1089, CB 1093 and KH 1060; retinoids such as retinoic acid; pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovorin.

Anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), raloxifene, megastrol, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LYL17018, onapristone, and FARESTON® toremifene; aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® megestrol acetate, AROMASIN® exemestane, formestanie, fadrozole, RIVISOR® vorozole, FEMARA® letrozole, and ARIMIDEX® anastrozole; and anti-androgens such as flutamide, bicalutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in abherant cell proliferation, such as, for example, PKC-alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; PROLEUKIN® rIL-2; LURTOTECAN® topoisomerase 1 inhibitor; ABARELIX® rmRH; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

In some embodiments, the additional method of treatment is radiation therapy. The compound described herein may be administered before, after, or at the same time as the radiation.

Compounds described herein may also be administered to a patient that has undergone or will undergo surgery as treatment for the cancer.

In a specific embodiment, the compound described herein is administered concurrently with the chemotherapeutic agent or with radiation therapy. In another specific embodiment, the chemotherapeutic agent or radiation therapy is administered prior or subsequent to administration of compound described herein, in one aspect at least an hour, five hours, 12 hours, a day, a week, a month, in further aspects several months (*e.g.*, up to three months), prior or subsequent to administration of a compound described herein.

A chemotherapeutic agent can be administered over a series of sessions. Any one or a combination of the chemotherapeutic agents listed herein or otherwise known in the art can be administered. With respect to radiation, any radiation therapy protocol can be used depending upon

the type of cancer to be treated. For example, but not by way of limitation, x-ray radiation can be administered; in particular, high-energy megavoltage (radiation of greater that 1 MeV energy) can be used for deep tumors, and electron beam and orthovoltage x-ray radiation can be used for skin cancers. Gamma-ray emitting radioisotopes, such as radioactive isotopes of radium, cobalt and other elements, can also be administered.

Additionally, methods of treatment of cancer with a compound described herein are provided as an alternative to chemotherapy or radiation therapy where the chemotherapy or the radiation therapy has proven or can prove too toxic, *e.g.*, results in unacceptable or unbearable side effects, for the subject being treated. Additionally, methods of treatment of cancer with a compound described herein are provided as an alternative to surgery where the surgery has proven or can prove unacceptable or unbearable for the subject being treated.

Provided are methods of treating cancer in a mammal comprising administering to a mammal in need thereof an effective amount of a compound or a pharmaceutical composition described herein.

Also provided are methods of increasing survival of a mammal having cancer, comprising administering to a mammal in need thereof an effective amount of a compound or a pharmaceutical composition described herein.

Also provided are methods of inhibiting tumor growth in a mammal comprising administering to a mammal in need thereof an effective amount of a compound or a pharmaceutical composition described herein.

Also provided are compounds and pharmaceutical compositions described herein for use in a method of treatment of the human or animal body by therapy.

Also provided are compounds and pharmaceutical compositions described herein for use in treating cancer in a mammal.

Also provided are compounds and pharmaceutical compositions described herein for use in increasing survival of a mammal having cancer.

Also provided are compounds and pharmaceutical compositions described herein for use in inhibiting tumor growth in a mammal.

Also provided are uses of a compound described herein in the manufacture of a medicament for treating cancer in a mammal.

Also provided are uses of a compound described herein in the manufacture of a medicament for increasing survival of a mammal having cancer.

Also provided are uses of a compound described herein in the manufacture of a medicament for inhibiting tumor growth in a mammal.

In some embodiments, the cancer is selected from: carcinomas, sarcomas, hematopoietic cancers, and tumors of neuroepithelial tissue.

Also provided are methods of treating an indication selected from: cancer, arthritis, arthrosis, multiple sclerosis, neural damage, cartilage damage, and psoriasis in a mammal comprising administering to a mammal in need thereof an effective amount of a compound or a pharmaceutical composition described herein.

Also provided are compounds and pharmaceutical compositions described herein for use in a method of treatment of an indication selected from: cancer, arthritis, arthrosis, multiple sclerosis, neural damage, cartilage damage, and psoriasis.

Also provided are uses of a compound described herein in the manufacture of a medicament for treating an indication selected from: cancer, arthritis, arthrosis, multiple sclerosis, neural damage, cartilage damage, and psoriasis.

Also provided are methods of treating an indication selected from: arthritis, multiple sclerosis, and psoriasis in a mammal comprising administering to a mammal in need thereof an effective amount of a compound or a pharmaceutical composition described herein.

Also provided are compounds and pharmaceutical compositions described herein for use in a method of treatment of an indication selected from: arthritis, multiple sclerosis, and psoriasis.

Also provided are uses of a compound described herein in the manufacture of a medicament for treating an indication selected from: arthritis, multiple sclerosis, and psoriasis.

ADMINISTRATION

Provided are pharmaceutical compositions comprising a compound described herein, and a pharmaceutically acceptable carrier, diluent or excipient.

For the purposes of administration, the compounds of the present disclosure may be administered as a raw chemical or may be formulated as pharmaceutical compositions. Pharmaceutical compositions of the present disclosure comprise a compound described herein and a pharmaceutically acceptable carrier, diluent or excipient. The compound described herein is present in the composition in an amount which is effective to treat a particular disease or condition of interest, *e.g.*, in an amount sufficient to treat cancer or tumor cell growth, and preferably with acceptable toxicity to the patient. The activity of compounds described herein can be determined by one skilled in the art, for example, as described in the Examples below. Appropriate concentrations and dosages can be readily determined by one skilled in the art.

Administration of the compounds described herein, or their pharmaceutically acceptable salts, in pure form or in an appropriate pharmaceutical composition, can be carried out via any of the accepted modes of administration of agents for serving similar utilities. The pharmaceutical compositions of the disclosure can be prepared by combining a compound described herein with an appropriate pharmaceutically acceptable carrier, diluent or excipient, and may be formulated into preparations in solid, semi solid, liquid or gaseous forms, such as tablets, capsules, powders, granules,

ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. Typical routes of administering such pharmaceutical compositions include, without limitation, oral, topical, transdermal, inhalation, parenteral, sublingual, buccal, rectal, vaginal, and intranasal. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. Pharmaceutical compositions of the disclosure are formulated so as to allow the active ingredients contained therein to be bioavailable upon administration of the composition to a patient. Compositions that will be administered to a subject or patient take the form of one or more dosage units, where for example, a tablet may be a single dosage unit, and a container of a compound described herein in aerosol form may hold a plurality of dosage units. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remington: The Science and Practice of Pharmacy (22nd ed.) eds. Loyd V. Allen, Jr., *et al.*, Pharmaceutical Press, 2012. The composition to be administered will, in any event, contain a therapeutically effective amount of a compound described herein, for treatment of a disease or condition of interest in accordance with the teachings of this disclosure.

A pharmaceutical composition described herein may be in the form of a solid or liquid. In one aspect, the carrier(s) are particulate, so that the compositions are, for example, in tablet or powder form. The carrier(s) may be liquid, with the compositions being, for example, an oral syrup, injectable liquid or an aerosol, which is useful in, for example, inhalatory administration.

When intended for oral administration, pharmaceutical compositions of the present disclosure typically are either solid or liquid form, where semi solid, semi liquid, suspension and gel forms are included within the forms considered herein as either solid or liquid.

As a solid composition for oral administration, the pharmaceutical compositions may be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer or the like form. Such a solid composition will typically contain one or more inert diluents or edible carriers. In addition, one or more of the following may be present: binders such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, gum tragacanth or gelatin; excipients such as starch, lactose or dextrins, disintegrating agents such as alginic acid, sodium alginate, Primogel, corn starch and the like; lubricants such as magnesium stearate or Sterotex; glidants such as colloidal silicon dioxide; sweetening agents such as sucrose or saccharin; a flavoring agent such as peppermint, methyl salicylate or orange flavoring; and a coloring agent.

When the pharmaceutical composition is in the form of a capsule, for example, a gelatin capsule, it may contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol or oil.

Pharmaceutical compositions described herein may be in the form of a liquid, for example, an elixir, syrup, solution, emulsion or suspension. The liquid may be for oral administration or for delivery by injection, as two examples. When intended for oral administration, pharmaceutical

compositions described herein typically contain, in addition to the present compounds, one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition intended to be administered by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent may be included.

Liquid pharmaceutical compositions described herein, whether they be solutions, suspensions or other like form, may include one or more of the following adjuvants: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. Parenteral preparations can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Physiological saline is a preferred adjuvant. An injectable pharmaceutical composition is preferably sterile.

A liquid pharmaceutical composition described herein intended for either parenteral or oral administration should contain an amount of a compound described herein such that a suitable dosage will be obtained.

Pharmaceutical compositions described herein may be intended for topical administration, in which case the carrier may suitably comprise a solution, emulsion, ointment or gel base. The base, for example, may comprise one or more of the following: petrolatum, lanolin, polyethylene glycols, bee wax, mineral oil, diluents such as water and alcohol, and emulsifiers and stabilizers. Thickening agents may be present in a pharmaceutical composition for topical administration. If intended for transdermal administration, the composition may include a transdermal patch or iontophoresis device.

Pharmaceutical compositions described herein may be intended for rectal administration, in the form, for example, of a suppository, which will melt in the rectum and release the drug. Compositions for rectal administration may contain an oleaginous base as a suitable nonirritating excipient. Such bases include, without limitation, lanolin, cocoa butter and polyethylene glycol.

Pharmaceutical compositions described herein may include various materials, which modify the physical form of a solid or liquid dosage unit. For example, the composition may include materials that form a coating shell around the active ingredients. The materials that form the coating shell are typically inert, and may be selected from, for example, sugar, shellac, and other enteric coating agents. Alternatively, the active ingredients may be encased in a gelatin capsule.

Pharmaceutical compositions described herein may be prepared in dosage units that can be administered as an aerosol. The term aerosol is used to denote a variety of systems ranging from those of colloidal nature to systems consisting of pressurized packages. Delivery may be by a liquefied or

compressed gas or by a suitable pump system that dispenses the active ingredients. Aerosols of compounds described herein may be delivered in single phase, bi phasic, or tri phasic systems in order to deliver the active ingredient(s). Delivery of the aerosol includes the necessary container, activators, valves, subcontainers, and the like, which together may form a kit. One skilled in the art, without undue experimentation may determine preferred aerosols.

The pharmaceutical compositions described herein may be prepared by methodology well known in the pharmaceutical art. For example, a pharmaceutical composition intended to be administered by injection can be prepared by combining a compound described herein with sterile, distilled water so as to form a solution. A surfactant may be added to facilitate the formation of a homogeneous solution or suspension. Surfactants are compounds that non covalently interact with the compound described herein so as to facilitate dissolution or homogeneous suspension of the compound in the aqueous delivery system.

The compounds described herein, or their pharmaceutically acceptable salts, are administered in a therapeutically effective amount, which will vary depending upon a variety of factors including the activity of the specific compound employed; the metabolic stability and length of action of the compound; the age, body weight, general health, sex, and diet of the patient; the mode and time of administration; the rate of excretion; the drug combination; the severity of the particular disorder or condition; and the subject undergoing therapy.

Compounds described herein, or pharmaceutically acceptable derivatives thereof, may also be administered simultaneously with, prior to, or after administration of one or more other therapeutic agents. Such combination therapy includes administration of a single pharmaceutical dosage formulation which contains a compound described herein and one or more additional active agents, as well as administration of the compound described herein and each active agent in its own separate pharmaceutical dosage formulation. For example, a compound described herein and the other active agent can be administered to the patient together in a single oral dosage composition such as a tablet or capsule, or each agent administered in separate oral dosage formulations. Where separate dosage formulations are used, the compounds described herein and one or more additional active agents can be administered at essentially the same time, *i.e.*, concurrently, or at separately staggered times, *i.e.*, sequentially; combination therapy is understood to include all these regimens.

EXAMPLES

The following Examples illustrate various methods of making compounds described herein, *i.e.*, compounds of Formula I and related formulae. It is understood that one skilled in the art may be able to make these compounds by similar methods or by combining other methods known to one skilled in the art. It is also understood that one skilled in the art would be able to make, in a similar manner as described below, other compounds of Formula I not specifically illustrated below by using the appropriate starting components and modifying the parameters of the synthesis as needed. In general, starting components may be obtained from sources such as Sigma Aldrich, Lancaster Synthesis, Inc., Maybridge, Matrix Scientific, TCI, and Fluorochem USA, *etc.* or synthesized according to sources known to those skilled in the art (see, for example, Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, 5th edition (Wiley, December 2000)) or prepared as described herein.

The following examples are provided for purposes of illustration, not limitation.

Example 1: Production of Truncated Recombinant VAR2CSA Proteins.

All protein truncations are produced according to previously defined domain borders (Dahlbäck *et al.* J Biol Chem 286: 15908-17). The CIDRPAM domain is divided into two domains ID2a and ID2b, where ID2a is the N-terminal part of CIDRPAM not containing the CIDR-like sequence and ID2b corresponds to the CIDR-like sequence. There are two DBL2X borders—DBL2Xa and DBL2Xb. DBL2Xb incorporates 93 amino acids of ID2a. Primers used in cloning are listed in Table 5. Fragments are expressed in baculovirus-infected insect cells or C3029H *E. coli* cells as soluble proteins as described in Methods 1 and 2. Most proteins are produced based on the FCR3 genotype. Some FCR3 fragments do not express and these are instead made based on the 3D7 genotype. VAR2CSA polypeptides from both genotypes bind equally to plCSA.

Table 5: Cloning Primers

FCR3 Primers

Protein	Forward Primer	Reverse Primer
ID1-ID2b	AACTACATCAAGGGCGAC (SEQ ID NO:76)	CTTGTTGATATTGGTGTCGGT (SEQ ID NO:77)
DBL1X-ID2a	CACAGCGATAGCGGCAAG (SEQ ID NO:78)	GTCCAGCTTGCTGGAGTT (SEQ ID NO:79)
ID1-ID2a	AACTACATCAAGGGCGAC (SEQ ID NO:80)	GTCCAGCTTGCTGGAGTT (SEQ ID NO:81)
ID1-DBL2Xa	AACTACATCAAGGGCGAC (SEQ ID NO:82)	AGCGGCGTTGGTGGTGGA (SEQ ID NO:83)
ID1-DBL2Xb	AACTACATCAAGGGCGAC (SEQ ID NO:84)	GTACTTGTACCGGTAGGG (SEQ ID NO:85)
DBL1X-DBL2Xb	CACAGCGATAGCGGCAAG (SEQ ID NO:86)	GTACTTGTACCGGTAGGG (SEQ ID NO:87)

5

3D7 Primers

Protein	Forward Primer	Reverse Primer
DBL2X-DBL4ε	CTGACCAACTGCTACAAG (SEQ ID NO:88)	GGTCCAGAGGGTACAGCTT (SEQ ID NO:89)
ID1-DBL3ε	CTGTCCTTCATCCTGAAC (SEQ ID NO:90)	TTCAGCGTTGTTGTACTCGTA (SEQ ID NO:91)
ID1-DBL4ε	CTGTCCTTCATCCTGAAC (SEQ ID NO:92)	GTCCAGAGGGTACAGCTT (SEQ ID NO:93)
DBL1X-ID2b	CACTCTGACTCTGGCACC (SEQ ID NO:94)	AGAGGACTTCATCTTGTTGTTGGT (SEQ ID NO:95)
ID1-ID2b	CTGTCCTTCATCCTGAAC (SEQ ID NO:96)	AGAGGACTTCATCTTGTTGTTGGT (SEQ ID NO:97)
DBL1X-ID2a	CACTCTGACTCTGGCACC (SEQ ID NO:98)	GTCCAGCTTAGAGGAGTT (SEQ ID NO:99)
ID1-ID2a	CTGTCCTTCATCCTGAAC (SEQ ID NO:100)	GTCCAGCTTAGAGGAGTT (SEQ ID NO:101)
DBL1X-DBL2Xa	CACTCTGACTCTGGCACC (SEQ ID NO:102)	GGCGGCGTTGGTGGTAGA (SEQ ID NO:103)
ID1-DBL2Xa	CTGTCCTTCATCCTGAAC (SEQ ID NO:104)	GGCGGCGTTGGTGGTAGA (SEQ ID NO:105)
DBL1X-DBL2Xb	CACTCTGACTCTGGCACC (SEQ ID NO:106)	GTACTTGTATCCGTGGGG (SEQ ID NO:107)
ID1-DBL2Xb	CTGTCCTTCATCCTGAAC (SEQ ID NO:108)	GTACTTGTATCCGTGGGG (SEQ ID NO:109)

10 **Mutating Putative plCSA Binding Sites**

	PCR1	
	Fragment 1	
Protein	Forward	Reverse
DBL1X-ID2a (DSM Deletion)	CACAGCGATAGCGGCAAG (SEQ ID NO:110)	GGTGTCGAAGTTGATGTCGGGCAGATTGCCCAGGTA (SEQ ID NO:111)
Alanine sub. K(626,629,630), R(631)	CACAGCGATAGCGGCAAG (SEQ ID NO:112)	AGCTGCGGCCAGATTAGCGCCCTCGTGGAAGGACAC (SEQ ID NO:113)
Alanine sub. K(459,460,461,464)	CACAGCGATAGCGGCAAG (SEQ ID NO:114)	AGCGCATTCAGCTGCGGCGTTGGTCTTGATGGAGCT (SEQ ID NO:115)
	Fragment 2	
Protein	Forward	Reverse
DBL1X-ID2a (DSM Deletion)	CACAGCGATAGCGGCAAG (SEQ ID NO:116)	GTCCAGCTTGCTGGAGTT (SEQ ID NO:117)
Alanine sub. K(626,629,630), R(631)	GCTAATCTGGCCGCAGCTTACCCCCAGAATAAGAAC (SEQ ID NO:118)	GTCCAGCTTGCTGGAGTT (SEQ ID NO:119)
Alanine sub. K(459,460,461,464)	GCCGCAGCTGAATGCGCTGACGTGAAGCTGGGCGTG (SEQ ID NO:120)	GTCCAGCTTGCTGGAGTT (SEQ ID NO:121)
	PCR2	
	Final Constuct	
Protein	Forward	Reverse
DBL1X-ID2a (DSM Deletion)	CACAGCGATAGCGGCAAG (SEQ ID NO:122)	GTCCAGCTTGCTGGAGTT (SEQ ID NO:123)
Alanine sub. K(626,629,630), R(631)	CACAGCGATAGCGGCAAG (SEQ ID NO:124)	GTCCAGCTTGCTGGAGTT (SEQ ID NO:125)
Alanine sub. K(459,460,461,464)	CACAGCGATAGCGGCAAG (SEQ ID NO:126)	GTCCAGCTTGCTGGAGTT (SEQ ID NO:127)

Method 1: Cloning and Protein Expression in Insect Cells.

VAR2CSA sequence fragments are amplified from codon optimized FCR3 (GenBank
15 accession no. GU249598) or 3D7 (GenBank accession no. 3Q247428) VAR2CSA genes using specific primers (Table 5). Simple fragments are amplified in a one-step PCR. Amino acid substitution constructs are made in a two-step PCR. First PCR amplifies two fragments from the codon optimized FCR3 template, containing overlapping complimentary ends. Second PCR amplifies

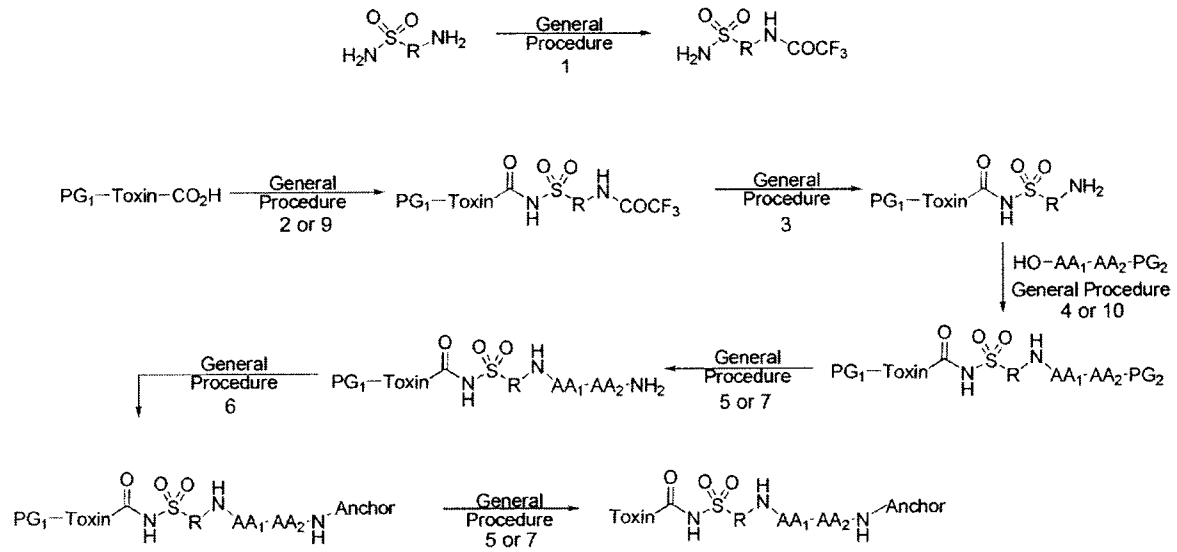
the total construct, using the two overlapping fragments as template with primers specific for the outer borders. All fragments are sequenced for verification. Fragments are cloned into the baculovirus vector pAcGP67-A (BD Biosciences), modified to contain a V5 and His tag at the C-terminal. The proteins are expressed in baculovirus-infected insect cells as soluble protein secreted into the cell culture supernatant. Briefly, linearized Bakpak6 Baculovirus DNA (BD Biosciences) is co-transfected with the pAcGP67-A plasmids, into Sf9 insect cells for generation of recombinant virus particles. 10 mL of the second amplification is used to infect High-Five cells in 400 mL serum-free medium (10486, GIBCO) at a density of 1×10^6 cells/mL. The secreted recombinant protein is harvested from the supernatant 3 days after initial infection. The supernatant is filtered (0.2 μm), dialyzed and concentrated before protein purification. The filtered supernatant containing the secreted recombinant protein is dialyzed using an AKTA cross-flow (GE Healthcare). The dialysis is performed in 10 mM NaH_2PO_4 (pH 7.4, Sigma-Aldrich) and 500 mM NaCl. The resulting solution is filtered (0.2 μm) and imidazole is added to a final concentration of 15 mM. The protein is then purified on a 1-mL HisSelect column (H8286, Sigma-Aldrich). Bound protein is eluted with 10 mM NaH_2PO_4 (pH 7.4), 500 mM NaCl, and 500 mM imidazole. The purity and structural integrity of the protein was verified by SDS-PAGE.

Method 2: Protein Expression in *E. coli* Cells

Recombinant VAR2CSA proteins were expressed in C3029H or C3030 *E. coli* SHuffle cells. 20 mL warm 2xYT [+AMP] medium was inoculated with an *E. coli* clone bearing an appropriate plasmid and incubated overnight at 37 °C overnight with shaking (150 rpm). 800 mL of prewarmed 2xYT [+AMP] in a 5 L flask was next inoculated with 16 mL of the starter culture and incubated at 37 °C with shaking (100-150 rpm) until OD600 reached 0.5-0.8, after which time the temperature was adjusted to 20 °C. After 20 minutes the culture was induced with 80 μL 1 M IPTG and incubated for a further 18-20 hours with shaking (100-150 rpm). Cells were harvested by centrifugation (10000 × g, 10 min) and the pellet was resuspended in 40 mL lysis buffer (10 mM $NaPO_4$ (pH 7.2), 0.5 M NaCl, 60 mM imidazole + CMPIT protease inhibitor tablet without EDTA per 20 mL buffer) and split equally into two 50 mL centrifuge tubes. Cells were lysed by sonication (2 × 5 mins.) on ice and the debris was removed by centrifugation (40000 × g, 30 min, 4 °C). Supernatants were filtered (0.2 μm) into a tube on ice and either purified directly or frozen at -20 °C.

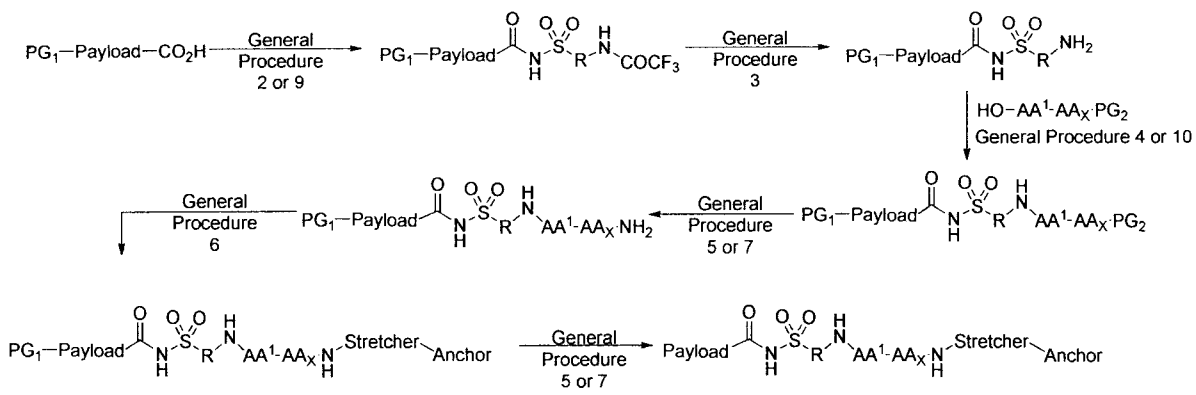
Example 2: Preparation of T-L^2-P^2.

Scheme 1



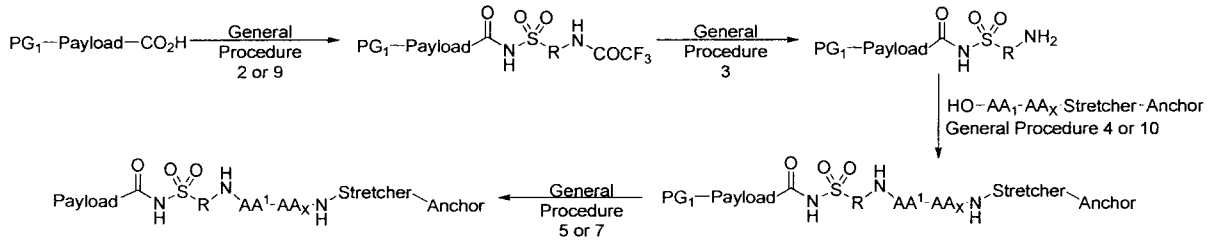
Scheme 1 illustrates a particular embodiment of a general scheme for the synthesis of L^2–P^2. In further embodiments, the protecting group (PG_1) is removed from the Toxin (P^2) before amino acid (*e.g.*, AA_1-AA_2) addition. In certain embodiments, the Anchor includes a functional group that can form a covalent bond with the target moiety (T). In other embodiments, the Anchor comprises a Stretcher.

Scheme 2



Scheme 2 illustrates a particular embodiment of a general scheme for the convergent synthesis of a P-L complex where the JPB between the payload and AA sequence is assembled prior to installation of stretcher and anchor moieties. This synthetic approach was used to generate the following compounds: Compound G, Compound H, Compound I, Compound J, Compound K, Compound L, Compound M, Compound N, Compound O, Compound P, Compound Q, Compound R, Compound U, Compound EE, Compound FF, Compound GG, Compound HH, Compound II, and Compound JJ.

Scheme 3



Scheme 3 illustrates a particular embodiment of a general scheme for the convergent synthesis of a P-L complex where the JPB is established between the payload and a proteolytic sequence that already contains a stretcher and anchor functionality. This synthetic approach was used to generate the following compounds: Compound S, Compound T, Compound W, Compound X, Compound Y, Compound Z, Compound AA, Compound BB, Compound CC, and Compound DD.

In certain embodiments, the general scheme comprises the procedures as discussed below. As will be understood by the reasonably skilled artisan, these procedures are illustrative of certain embodiments, and could be performed with alternative solvents, reagents and protecting groups known to be suitable in the art.

Example 2.1: General Procedure 1 — Trifluoroacetamide Installation.

To a stirred suspension of the amine in 1,4-dioxane was added trifluoroacetic anhydride (1.1 equivalents). The reaction mixture transitioned from a suspension to a solution and back to a suspension again. The progress of the reaction was monitored by TLC and/or HPLC-MS for completion. Once the starting material was fully consumed, the reaction was diluted with hexanes or diethyl ether, filtered on a Buchner funnel and the resulting solids were dried under reduced pressure to give the pure trifluoroacetamide.

Example 2.2: General Procedure 2 — DCC/DMAP Mediated *N*-acyl Sulfonamide Formation.

To a stirred solution of the acid in dichloromethane was added a solution of the sulfonamide (1.3 equivalents, in dichloromethane, *N,N*-dimethylformamide, or a mixture thereof, as necessary).

Dicyclohexylcarbodiimide (1.2 equivalents) was added and subsequently *N,N*-dimethylaminopyridine (1.2 equivalents). Reaction course was monitored by HPLC-MS (typically 16 h) and excess by-products could be precipitated by the addition of diethyl ether. Solids were removed by filtration and washed with 1:1 diethyl ether/dichloromethane. The combined organic layers were concentrated, and the residue was purified by silica gel chromatography to give the desired *N*-acyl sulfonamide.

Example 2.3: General Procedure 3 — Trifluoroacetamide Saponification.

To a solution of the trifluoroacetamide containing-construct in 1,4-dioxane or methanol was added lithium hydroxide (10 equivalents) and water (10% v/v). The reaction was allowed to stir at room temperature or optionally heated to 50°C. Reaction course was monitored by HPLC-MS. Upon completion, volatiles were removed under reduced pressure and the aqueous layer was quenched with an aqueous solution of 5% w/v citric acid or 1 M hydrochloric acid. The resulting aqueous solution was washed successively with dichloromethane or ethyl acetate and the organic phases were pooled, dried over $MgSO_4$, filtered and concentrated. The reaction product was either used "as is" or purified by silica gel chromatography as necessary.

Example 2.4: General Procedure 4 — HATU Mediated Peptide Bond Formation.

To a stirred solution of the carboxylic acid in a minimal amount of dichloromethane or N,*N*-dimethylformamide or mixture thereof, at 0°C was added HATU (1.05-1.2 equivalents) and either N,*N*-diisopropylamine (2-4 equivalents) or 2,4,6-collidine (2-4 equivalents). Stirring was continued for a brief induction period (5-20 minutes) at which time the reaction was charged with a solution of the amine in dichloromethane. The reaction was allowed to warm to room temperature and monitored for progress by HPLC-MS. Upon completion, volatiles were removed under reduced pressure and the residual material was purified by silica gel chromatography or reverse phase HPLC to furnish amide in adequate purity.

Example 2.5: General Procedure 5 — Fmoc Group Removal.

The Fmoc-protected compound was dissolved in 20% piperidine in *N,N*-dimethylformamide. The reaction course was monitored by HPLC-MS. When complete, all volatiles were removed under reduced pressure to yield a residue that was either purified by silica gel chromatography or used directly in the next step.

Example 2.6: General Procedure 6 — *N*-Acylation of Amines Using NHS-activated Esters.

To a solution of the amine in a minimal amount of *N,N*-dimethylformamide was added the corresponding *N*-hydroxy succinimide containing ester (1.5 equivalents). The progress of the reaction was monitored by HPLC-MS (typically ~16h) at which point all volatiles were removed under reduced pressure. The residue was then purified by either silica gel chromatography or reverse phase HPLC to give the desired amide product.

Example 2.7: General Procedure 7 — Boc group Removal.

To a solution of the Boc-protected compound in dichloromethane was added 10% v/v trifluoroacetic acid. Reaction course was monitored by HPLC-MS. Upon reaction completion, all volatiles were removed under reduced pressure. The residual material was purified either by reverse phase HPLC, silica gel chromatography or precipitation from a mixture of cold methanol/dichloromethane/diethyl ether.

Example 2.7.1: General Procedure 8 — 4-Anilino Sulfonamide Synthesis.

To a stirred suspension or solution of the starting aniline in CH_2Cl_2 (0.1 M) was added trifluoroacetic anhydride (1.1 equiv). The reaction was allowed to stir for ~1h at which point it was concentrated under reduced pressure. The residue was twice dissolved in $CHCl_3$ and concentrated to give the desired trifluoroacetanilide in quantitative yield with the expected analytical results.

The trifluoroacetanilide (~8mmol) was dissolved in $CHCl_3$ (10 mL). Chlorosulfonic acid (3 equiv) was added with stirring. The resulting solution was heated to 70°C for 1h, then cooled to room temperature at which time thionyl chloride (2 equiv) was added with stirring. The resulting biphasic mixture was re-heated to 70°C for 15 minutes. The reaction mixture was then twice diluted with $CHCl_3$ and concentrated *in vacuo* to remove excess acids.

The resulting phenylchlorosulphonic acid was dissolved in 1,4-dioxane (~10 mL) and the resulting solution was added dropwise to a concentrated solution of aqueous ammonia (10 mL) at 0°C with vigorous stirring. The reaction was quenched by addition of 1M citric acid and adjusted to pH = 3. In most cases the sulfonamide precipitated and was filtered directly from the aqueous phase; in instances where the product did not precipitate, the reaction was diluted with ethyl acetate (~100 mL), transferred to a separatory funnel and the organic phase was washed with brine before being dried over $MgSO_4$ and concentrated to give the desired 4-trifluoroacetanilide substituted sulfonamides.

Example 2.7.2: General Procedure 9 — Alternative Acyl Benzotriazole Mediated *N*-Acyl Sulfonamide Formation.

This procedure was adapted from the one described in ARKIVOC 2004 (xii), 14-22.

Example 2.7.3: General Procedure 10 — EDCI/Cu(II) Mediated Peptide Bond Formation.

To a stirred solution of the carboxylic acid in a minimal amount of 30% *N,N*-dimethylformamide in dichloromethane was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.95 equiv), 1-hydroxy-7-azabenzotriazole (1.0 equiv), the amine (0.33 equiv) and anhydrous copper (II) chloride (1.0 equiv) in sequence with a brief pause between each additional reagent. Stirring was continued at room temperature and progress of the reaction was monitored by HPLC-MS. Upon completion, volatiles were removed under reduced pressure and the residual material was purified by silica gel chromatography or reverse phase HPLC to furnish the desired amide in adequate purity.

Example 2.7.4: General Procedure 11 — Ester Saponification.

To a solution of the ester containing compound in 1,4-dioxane or methanol was added lithium hydroxide (10 equivalents) and water (10% v/v). The reaction was allowed to stir at room temperature or optionally heated to 50 °C. Reaction course was monitored by HPLC-MS. Upon completion, volatiles were removed under reduced pressure, the aqueous layer was pH adjusted if necessary and washed successively with dichloromethane or ethyl acetate. The organic phases were pooled, dried over MgSO4, filtered and concentrated. The reaction product was either used "as is" or purified by silica gel chromatography as necessary.

Example 2.8.1: Fmoc-Val-Cit-OH: (*R*)-2-((*R*)-2-(((9*H*-Fluoren-9-yl-methoxy)carbonylamino)-3-methylbutanamido)-5-ureidopentanoic acid, Fmoc-Valine-Citrulline-OH.

The title compound was prepared according to Dubowchik *et al.*, Bioconjugate Chem., 2002, 13, 855-869. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.56 (s, 1H), 8.21 (d, J = 7.3 Hz, 1H), 7.90 (d, J = 7.5 Hz, 2H), 7.76 (t, J = 7.0 Hz, 2H), 7.49-7.39 (m, 3H), 7.38-7.23 (m, 2H), 5.96 (t, J = 5.9 Hz, 1H), 5.40 (s, 2H), 4.34-4.09 (m, 4H), 3.93 (dd, J = 9.1, 7.1 Hz, 1H), 3.39 (q, J = 7.0 Hz, 3H), 2.96 (q, J = 6.5 Hz, 2H), 1.97 (d, J = 6.9 Hz, 1H), 1.86-1.63 (m, 1H), 1.57 (dtd, J = 13.9, 9.0, 5.4 Hz, 1H), 1.41 (dhept, J = 13.2, 6.9 Hz, 2H), 0.88 (dd, J = 13.3, 6.7 Hz, 6H). $C_{26}H_{32}N_4O_6$ calcd. $[M+H]^+$ 497.23. found $[M+H]^+$ 497.19.

Example 2.8.2: Fmoc-Val-Cit-OH: (*S*)-2-((*S*)-2-(((9*H*-Fluoren-9-yl-methoxy)carbonylamino)-3-methylbutanamido)-5-ureidopentanoic acid, Fmoc-Valine-Citrulline-OH.

The title compound was prepared according to Dubowchik *et al.*, Bioconjugate Chem., 2002, 13, 855-869. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.56 (s, 1H), 8.21 (d, J = 7.3 Hz, 1H), 7.90 (d, J = 7.5 Hz, 2H), 7.76 (t, J = 7.0 Hz, 2H), 7.49-7.39 (m, 3H), 7.38-7.23 (m, 2H), 5.96 (t, J = 5.9 Hz, 1H), 5.40 (s, 2H), 4.34-4.09 (m, 4H), 3.93 (dd, J = 9.1, 7.1 Hz, 1H), 3.39 (q, J = 7.0 Hz, 3H), 2.96 (q, J = 6.5 Hz, 2H), 1.97 (d, J = 6.9 Hz, 1H), 1.86-1.63 (m, 1H), 1.57 (dtd, J = 13.9, 9.0, 5.4 Hz, 1H), 1.41 (dhept, J = 13.2, 6.9 Hz, 2H), 0.88 (dd, J = 13.3, 6.7 Hz, 6H).). $C_{26}H_{32}N_4O_6$ calcd. $[M+H]^+$ 497.23. found $[M+H]^+$ 497.19.

Example 2.9: MC-NHS: 2,5-Dioxopyrrolidin-1-yl 6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-l-yl)hexanoate.

To a stirred solution of 6-aminocaproic acid (10.0 g, 76.2 mmol, 1.0 eq) in acetic acid (75 mL), maleic anhydride (7.85 g, 80.0 mmol, 1.05 eq) was added. The solids took a few minutes to dissolve, then after ca. 5 min, white solids began to crash out. After an hour, the suspension thickened to a white cake. This material was scooped onto a fritted funnel and washed with toluene and dried *in vacuo* with heating to remove all traces of acetic acid. The intermediate powder was taken up in toluene (250 mL), triethylamine (21.3 mL, 152 mmol, 2.0 eq) was added, and the mixture heated to reflux with a Dean—Stark trap. After 5 h of reflux, the mixture was cooled and the clear toluene layer was decanted from the rest of the sticky residue in the flask. The toluene was removed *in vacuo* to yield a triethylamine salt of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoate. The salt was redissolved in toluene, and a small amount of acetic acid was added, then concentrated. Next, the mixture was taken up in 50% saturated sodium bicarbonate, and 1 M HCl was added to adjust the pH to 3, forming a milky precipitate. This was extracted three times with EtOAc, combined organics dried over sodium sulfate, filtered, and concentrated *in vacuo* to yield pure 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoate (3.08 g, 19%). To a stirred solution of 6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoate (3.08 g, 14.6 mmol, 1.0 eq) and *N*-hydroxysuccinimide (1.76 g, 15.3 mmol, 1.05 eq) in EtOAc (30 mL) at 0 °C, was added dicyclohexylcarbodiimide (3.16 g, 15.3 mmol, 1.05 eq). The reaction was then allowed to warm to rt. After 20 h, the reaction was filtered and washed with EtOAc and the filtrate concentrated. The residue was purified by flash chromatography to yield the title compound (2.16 g, 48%) as a clear oil that solidified slowly to a waxy white solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.71 (s, 2H), 3.56 (t, J = 7.2 Hz, 2H), 2.86 (s, 4H), 2.63 (t, J = 7.4 Hz, 2H), 1.80 (p, J = 7.4 Hz, 2H), 1.73-1.57 (m, 2H), 1.50-1.35 (m, 2H). *m/z* calcd. for $C_{14}H_{16}N_2O_6$ = 308.10. Found $[M+H]^+$ = 309.13. R_f = 0.28 (50% EtOAc/Hex).

Example 2.10: Fmoc-Phe-Lys(Boc)-OH: (*R*)-2((*R*)-2(((9*H*-Fluoren-9-yl-methoxy)carbonylamino)-3-phenylpropanamido)-6-(*tert*-butoxycarbonylamino)hexanoic Acid; Fmoc-Phenylalanine-Lysine(Boc)-OH.

The title compound was prepared according to Walker *et al.*, Bioorganic Med Chem Lett, 2004, 14, 4323-4327. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (d, J = 7.7 Hz, 1H), 7.89 (d, J = 7.6 Hz, 2H), 7.71-7.57 (m, 2H), 7.41 (td, J = 7.6, 3.8 Hz, 2H), 7.33 (t, J = 7.5 Hz, 2H), 7.30-7.23 (m, 4H), 7.19 (t, J = 7.3 Hz, 1H), 6.79 (t, J = 5.6 Hz, 1H), 4.37-4.24 (m, 1H), 4.24-4.07 (m, 5H), 3.02 (dd, J = 13.8, 3.5 Hz, 1H), 2.95-2.83 (m, 2H), 2.83-2.71 (m, 1H), 1.82-1.68 (m, 1H), 1.68-1.51 (m, 1H), 1.46-1.22 (m, 13H). *m/z* calcd. for $C_{35}H_{41}N_3O_7$ = 615.29. Found $[M+H]^+$ = 616.27, $[M-Boc+2H]^+$ = 516.16.

Example 2.11: MT-OH: 3-(2-(2-(2-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethoxy)ethoxy)ethoxy)propanoic Acid.

The title compound was prepared according to Warnecke, A., Kratz, F. Bioconjugate Chemistry 2003, 14, 377-387. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.74 (s, 2H), 3.87-3.72 (m, 4H), 3.72-3.62 (m, 10H), 2.73-2.64 (m, 2H). *m/z* calcd. for $C_{13}H_{29}NO_7$ = 301.12. Found $[M+H]^+$ = 302.14.

Example 2.12: MT-NHS: 2,5-Dioxopyrrolidin-1-yl 3-(2-(2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethoxy)ethoxy)propanoate.

MT-OH (2.6 g, 8.6 mmol, 1.0 eq) was treated with dicyclohexylcarbodiimide (1.87 g, 9.06 mmol, 1.05 eq), and *N*-hydroxysuccinimide (1.04 g, 6.06 mmol, 1.05 eq) in 30 mL of 5:1 EtOAc/dioxane at rt. After 36 h, the mixture was filtered, washing with EtOAc, and the residue was purified by flash chromatography to yield the title compound (309 mg, 9.0%) as a clear oil along with starting material (1.31 g, 50% recovered). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.72 (s, 2H), 3.87 (t, *J* = 6.4 Hz, 2H), 3.74 (t, *J* = 5.6 Hz, 2H), 3.70-3.58 (m, 10H), 2.93 (t, *J* = 6.4 Hz, 2H), 2.86 (s, 4H), 1.32-1.19 (m, 2H). *m/z* calcd. for $C_{17}H_{22}N_2O_9$ = 398.13. Found $[M+H]^+$ = 399.15, $[M+Na]^+$ = 421.14. R_f = 0.59 (10% (5% AcOH/MeOH)/10% Hex/CH_2Cl_2).

Example 2.13: Boc-HTI-286-OH: (6*S*,9*S*,12*S*,*E*)-9-*tert*-Butyl-12-isopropyl-2,2,5,11,14-pentamethyl-4,7,10-trioxo-6-(2-phenylpropan-2-yl)-3-oxa-5,8,11-triazapentadec-13-en-15-oic Acid.

The title compound was prepared according to Nieman *et al.* J. Nat. Prod. 2003, 66, 183-199. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.57 (d, *J* = 7.3 Hz, 2H), 7.48 (t, *J* = 7.8 Hz, 2H), 7.38 (t, *J* = 7.3 Hz, 1H), 6.80 (dq, *J* = 9.8, 1.6 Hz, 1H), 5.08 (t, *J* = 10.2 Hz, 1H), 4.95 (s, 1H), 4.37 (s, 1H), 3.17 (s, 3H), 2.53 (s, 3H), 2.15-2.02 (m, 1H), 1.94 (d, *J* = 1.5 Hz, 3H), 1.50 (s, 3H), 1.41 (s, 3H), 1.10 (s, 9H), 0.93 (d, *J* = 6.6 Hz, 3H), 0.92 (d, *J* = 6.6 Hz, 3H). $C_{32}H_{51}N_3O_6$ calcd. $[M+H]^+$ 574.38. found $[M+Na]^+$ 586.42, $[M+H]^+$ 574.46, $[M-Boc+2H]^+$ 474.39.

Example 2.14: Preparation of (*S*,*E*)-*N*-(4-(((*S*)-2-((*S*)-2-(6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)methyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound G).

Step 1: 4-(Azidomethyl)benzenesulfonamide.

To a stirred solution of 4-(bromomethyl)benzenesulfonamide (0.50 g) in *N*,*N*-dimethylformamide (1 mL) was added sodium azide (0.20 g). The suspension was heated to 50 °C for 3 hours at which point the solvent was removed under reduced pressure. The residue was partitioned between ethyl acetate and water. The organic phase was washed with brine, dried over magnesium

sulfate, filtered and concentrated to dryness to give the title compound as a syrup that solidified on standing. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.06-7.91 (m, 2H), 7.58-7.44 (m, 2H), 4.96 (s, 2H), 4.48 (s, 2H).

Step 2: 4-(Aminomethyl)benzenesulfonamide.

To a solution of 4-(azidomethyl)benzenesulfonamide (0.354g) in methanol (10 mL) in a round bottom flask equipped with a magnetic stirrer was added 10% Pd/C (0.05 g). The flask was evacuated of gases at reduced pressure and charged with hydrogen. This evacuation and charge was repeated three times at which point the suspension was left to stir overnight. At 16 h, TLC analysis indicated complete consumption of the starting material. The reaction was diluted with methanol (40 mL), Celite® was added and the mixture was filtered through a fritted glass funnel. The resulting solution was concentrated to dryness. ^{1}H NMR suggested that the material was sufficiently clean at this stage for further use without purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.77 (m, 2H), 7.53 (m, 2H), 5.76 (s, 2H), 3.76 (d, J = 11.9 Hz, 2H).

Step 3: 2,2,2-Trifluoro-*N*-(4-sulfamoylbenzyl)acetamide.

The title compound was synthesized by reaction of 4-(aminomethyl)benzenesulfonamide with TFAA according to General Procedure 1, with a ^{1}H NMR spectrum that was complicated by rotamers. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91-7.75 (m, 2H), 7.55-7.31 (m, 4H), 4.72 (m, 2H), 4.47 (d, J = 6.0 Hz, 1H), 3.18 (s, 2H).

Step 4: *tert*-Butyl (*S*)-1-((*S*)-1-(((*S*,*E*)-2,5-Dimethyl-6-oxo-6-(4-((2,2,2-trifluoroacetamido)methyl)phenylsulfonamido)hex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate).

The title compound was synthesized from Boc-HTI-286-OH according to General Procedure 2. ^{1}H NMR (400 MHz, Methanol-*d*4) δ 8.11-7.99 (m, 2H), 7.50 (dd, J = 18.3, 7.9 Hz, 4H), 7.39-7.07 (m, 7H), 6.43 (d, = 9.0 Hz, 1H), 5.17 (s, 1H), 4.68 (d, J = 8.9 Hz, 1H), 4.56 (s, 2H), 3.00 (d, J = 33.9 Hz, 3H), 2.88 (d, J = 7.6 Hz, 3H), 2.34 (s, 2H), 2.00 (d, J = 13.6 Hz, 1H), 1.81 (d, J = 6.4 Hz, 3H), 1.43 (s, 13H), 0.98-0.68 (m, 14H). $C_{41}H_{58}F_3N_5O_8S$ calcd. $[M+H]^+$ 838.40; found $[M+Na]^+$ 860.48; $[M+H]^+$ 838.46; $[M\text{-Boc}+2H]^+$ 738.33.

Step 5: (*S*,*E*)-*N*-(4-(Aminomethyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

The title compound was prepared from *tert*-butyl (*S*)-1-((*S*)-1-((*S*,*E*)-2,5-dimethyl-6-oxo-6-(4-((2,2,2-trifluoroacetamido)methyl)phenylsulfonamido)hex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate) according to General Procedures 3 and 7. ^{1}H NMR (400 MHz, Methanol-*d*4) δ 8.13 (d, J = 8.3 Hz, 2H), 7.68 (d, J = 8.4 Hz, 2H), 7.59-7.41 (m, 4H), 7.37 (t, J = 7.3 Hz, 1H), 6.51 (dd, J = 9.4, 1.7 Hz, 1H), 5.01 (t, J = 9.9 Hz, 1H), 4.37 (s, 1H), 4.24 (s, 2H), 3.17 (s, 3H), 2.51 (s, 3H), 2.12-1.96 (m, 1H), 1.84 (d, J = 1.5 Hz, 3H),

1.47 (s, 3H), 1.37 (s, 3H), 1.07 (s, 9H), 0.91 (m, 6H). $C_{34}H_{51}N_5O_5S$ calcd. $[M+H]^+$ 642.38; found $[M+H]^+$ 642.40.

Step 6: (9*H*-Fluoren-9-yl)methyl (*S*)-1-((*S*)-1-(4-(*N*-(*S*,*E*)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enoyl)sulfamoyl)benzylamino)-1-oxo-5-ureidopentan-2-ylamino)-3-methyl-1-oxobutan-2-ylcarbamate.

Synthesized from (*S*,*E*)-*N*-(4-(aminomethyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide and Fmoc-Val-Cit-OH according to General Procedure 4 with minor contamination by DIPEA and AcOH. Material used "as is" in the subsequent step. $C_{60}H_{81}N_9O_{10}S$ calcd. $[M+H]^+$ 1120.58; found $[M+H]^+$ 1120.68.

Step 7: (*S*,*E*)-*N*-(4-(((*S*)-2-((*S*)-2-Amino-3-methylbutanamido)-5-ureidopentanamido)methyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

The title compound was synthesized staring with (9*H*-fluoren-9-yl)methyl (*S*)-1-((*S*)-1-(4-(*N*-(*S*,*E*)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enoyl)sulfamoyl)benzylamino)-1-oxo-5-ureidopentan-2-ylamino)-3-methyl-1-oxobutan-2-ylcarbamate according to General Procedure 5.

Step 8: (*S*,*E*)-*N*-(4-(((*S*)-2-((*S*)-2-(6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)methyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

The title compound was synthesized from (*S*,*E*)-*N*-(4-(((*S*)-2-((*S*)-2-amino-3-methylbutanamido)-5-ureidopentanamido)methyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide and MC-NHS according to General Procedure 6. ^{1}H NMR (600 MHz, Methanol-*d*4) δ 7.89 (d, *J* = 8.0 Hz, 2H), 7.53-7.47 (m, 2H), 7.39 (t, *J* = 7.5 Hz, 4H), 7.28 (t, *J* = 7.3 Hz, 1H), 6.82 (s, 2H), 6.67 (d, *J* = 9.3 Hz, 1H), 5.03 (t, *J* = 10.0 Hz, 1H), 4.51-4.35 (m, 3H), 4.18 (d, *J* = 7.4 Hz, 1H), 3.65 (s, 1H), 3.50 (t, *J* = 7.1 Hz, 2H), 3.31 (s, 3H), 3.20-3.01 (m, 5H), 2.35-2.18 (m, 5H), 2.08 (dq, *J* = 13.9, 6.9 Hz, 1H), 2.02-1.91 (m, 6H), 1.91-1.77 (m, 4H), 1.72 (dtd, *J* = 14.0, 9.3, 5.2 Hz, 1H), 1.66-1.40 (m, 10H), 1.37 (s, 3H), 1.34-1.24 (m, 3H), 1.03 (s, 9H), 0.96 (dd, *J* = 6.8, 4.0 Hz, 6H), 0.91-0.86 (m, 3H), 0.84 (d, *J* = 6.6 Hz, 3H). $C_{55}H_{82}N_{10}O_{11}S$ calcd. $[M+H]^+$ 1091.59; found $[M+H]^+$ 1091.67.

Example 2.15: Preparation of (*S*,*E*)-*N*-(4-(((*R*)-6-amino-2-((*R*)-2-(6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-phenylpropanamido)hexanamido)methyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound H).

Step 1: *tert*-Butyl (*S*)-1-((*S*)-1-(((*S,E*)-6-(4-(Aminomethyl)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate.

The title compound was prepared from *tert*-butyl (*S*)-1-((*S*)-1-((*S,E*)-2,5-dimethyl-6-oxo-6-(4-((2,2,2-trifluoroacetamido)methyl)phenylsulfonamido)hex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate) according to General Procedure 3. See above for characterization.

Step 2: *tert*-Butyl (*S*)-1-(((*S*)-1-(((*S,E*)-6-(4-((5*R*,8*R*)-5-Benzyl-8-(4-((*tert*-butoxycarbonyl)amino)butyl)-1-(9*H*-fluoren-9-yl)-3,6,9-trioxo-2-oxa-4,7,10-triazaundecan-11-yl)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate (Compound H-1)

The title compound was prepared from *tert*-butyl (*S*)-1-(((*S*)-1-(((*S,E*)-6-(4-(aminomethyl)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate and Fmoc-Phe-Lys(Boc)-OH according to General Procedure 4. $C_{74}H_{98}N_8O_{13}S$ calcd. *m/z* = 1338.70 amu; found $[M+H]^+$ = 1339.86, $[M+Na]^+$ = 1361.88, $[M+K]^+$ = 1377.95, $[M\text{-Boc}+2H]^+$ = 1239.83, $[M\text{-}2Boc+3H]^+$ = 1139.72.

Step 3: *tert*-Butyl (*S*)-1-(((*S*)-1-(((*S,E*)-6-(4-(((*R*)-2-((*R*)-2-Amino-3-phenylpropanamido)-6-((*tert*-butoxycarbonyl)amino)hexanamido)methyl)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate (Compound H-2).

The title compound was prepared from Compound H-1 according to General Procedure 5. $C_{59}H_{88}N_8O_{11}S$ calcd. *m/z* = 1116.63 amu; found $[M+H]^+$ = 1117.78, $[M+Na]^+$ = 1139.80, $[M\text{-Boc}+2H]^+$ = 1017.72, $[M\text{-}2Boc+3H]^+$ = 917.64.

Step 4: *tert*-Butyl (*S*)-1-(((*S*)-1-(((*S,E*)-6-(4-(((*R*)-6-((*tert*-Butoxycarbonyl)amino)-2-((*R*)-2-(6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-phenylpropanamido)hexanamido)methyl)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate (Compound H-3).

The title compound was prepared from Compound H-2 and MC-NHS according to General Procedure 6. $C_{69}H_{99}N_9O_{14}S$ calcd. *m/z* = 1309.70 amu; found $[M+H]^+$ = 1310.89, $[M+Na]^+$ = 1332.91, $[M\text{-Boc}+2H]^+$ = 1210.86, $[M\text{-}2Boc+3H]^+$ = 1110.77.

Step 5: (*S,E*)-*N*-(4-(((*R*)-6-Amino-2-((*R*)-2-(6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-phenylpropanamido)hexanamido)methyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

The title compound was prepared from Compound H-3 according to General Procedure 7. $C_{59}H_{83}N_9O_{10}S$ calcd. *m/z* = 1109.60 amu; found $[M+H]^+$ = 1110.76, $[M+Na]^+$ = 1132.75, $[(M+2H)/2]^{2+}$ = 556.11.

Example 2.16: Preparation of (*S*,*E*)-*N*-(4-(((*S*)-2-((*S*)-2-(6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)methylbenzyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound I).

Step 1: 2,2,2-Trifluoro-*N*-(4-(sulfamoylmethyl)benzyl)acetamide.

The title compound was synthesized from commercially available (4-(aminomethyl)phenyl)methanesulfonamide and TFAA using General Procedure 1. ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.05 (s, 1H), 7.48-7.40 (m, 2H), 7.40-7.32 (m, 2H), 6.17 (s, 1H), 4.56 (d, *J* = 6.1 Hz, 2H), 4.35 (s, 2H).

Step 2: (*S*,*E*)-2,5-Dimethyl-*N*-((4-((2,2,2-trifluoroacetamido)methyl)benzyl)sulfonyl)-4-((*S*)-N,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound I-1).

The title compound was synthesized from Boc-HTI-286-OH and 2,2,2-trifluoro-*N*-(4-(sulfamoylmethyl)benzyl)acetamide according to General Procedure 2. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.49 (d, *J* = 7.7 Hz, 2H), 7.41-7.27 (m, 5H), 7.21 (d, *J* = 8.0 Hz, 2H), 6.36 (d, *J* = 9.4 Hz, 1H), 5.18 (s, 1H), 4.99 (s, 2H), 4.69 (s, 3H), 4.46 (s, 3H), 3.06-2.91 (m, 3H), 2.88 (d, *J* = 4.7 Hz, 3H), 2.04 (d, *J* = 1.8 Hz, 1H), 1.88 (d, *J* = 13.5 Hz, 3H), 1.79-1.69 (m, 1H), 1.68-1.57 (m, 1H), 1.52 (d, *J* = 8.2 Hz, 3H), 1.44 (s, 9H), 1.23-1.12 (m, 1H), 0.97 (t, *J* = 7.4 Hz, 1H), 0.90 (d, *J* = 6.0 Hz, 9H), 0.80 (d, *J* = 6.8 Hz, 3H). $C_{42}H_{60}F_3N_5O_8S$ calcd. *m/z* = 851.41 amu; found $[M+H]^+$ = 852.47, $[M+Na]^+$ = 874.47, $[M-Boc+2H]^+$ = 752.38.

Step 3: (*S*,*E*)-*N*-((4-(Aminomethyl)benzyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide(Compound I-2).

The title compound was prepared from Compound I-1 according to General Procedure 3. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.49 (t, *J* = 8.0 Hz, 2H), 7.40-7.30 (m, 4H), 7.28 (d, *J* = 7.9 Hz, 2H), 7.22 (q, *J* = 7.9 Hz, 1H), 6.48 (d, *J* = 9.4 Hz, 1H), 5.19 (s, 1H), 5.07-4.94 (m, 2H), 4.72 (s, 1H), 4.48 (s, 2H), 3.77 (s, 2H), 3.05-2.82 (m, 3H), 1.92-1.82 (m, 4H), 1.58-1.32 (m, 16H), 0.97-0.85 (m, 12H), 0.85-0.74 (m, 4H). $C_{40}H_{61}N_5O_7S$ calcd. *m/z* = 755.43 amu; found $[M+H]^+$ = 756.46, $[M+Na]^+$ = 778.48, $[M-Boc+2H]^+$ = 656.39.

Step 4: (*S*,*E*)-N-((4-(((*S*)-2-((*S*)-2-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino-3-methylbutanamido)-5-ureidopentanamido)methyl)benzyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-

trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound I-3).

The title compound was prepared from Compound I-2 and Fmoc-Val-Cit-OH according to General Procedure 4. $C_{66}H_{91}N_9O_{12}S$ calcd. *m/z* = 1233.65 amu; found $[M+H]^+$ = 1234.82, $[M+Na]^+$ = 1256.80, $[M-Boc+2H]^+$ = 1134.73.

Step 5: (*S*,*E*)-*N*-((4-(((*S*)-2-((*S*)-2-amino-3-methylbutanamido)-5-ureidopentanamido)methyl)benzyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound I-4).

The title compound was prepared from Compound I-3 according to General Procedure 5. $C_{51}H_{81}N_9O_{10}S$ calcd. *m/z* = 1011.58 amu; found $[M+H]^+$ = 1012.72, $[M+Na]^+$ = 1034.68, $[M-Boc+2H]^+$ = 912.66.

Step 6: (*S*,*E*)-*N*-((4-(((*S*)-2-((*S*)-2-((5-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)pentyl)amino)-3-methylbutanamido)-5-ureidopentanamido)methyl)benzyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound I-5).

The title compound was prepared from Compound I-4 and MC-NHS according to General Procedure 6. $C_{61}H_{92}N_{10}O_{13}S$ calcd. *m/z* = 1204.66 amu; found $[M+H]^+$ = 1205.84, $[M+Na]^+$ 1227.82, $[M-Boc+2H]^+$ = 1105.75.

Step 7: (*S*,*E*)-*N*-(4-(((*S*)-2-((*S*)-2-(6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)methylbenzyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

The title compound was prepared from Compound I-5 according to General Procedure 7. $C_{56}H_{84}N_{10}O_{11}S$ calcd. *m/z* = 1104.60 amu; found $[M+H]^+$ = 1105.78, $[M+Na]^+$ =1127.76, $[(M+2H)/2]^{2+}$ = 553.60.

Example 2.17: Preparation of (*S*,*E*)-*N*-(4-(((*R*)-6-Amino-2-((*R*)-2-(6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-phenylpropanamido)hexanamido)methyl)benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound J).

Step 1: (*S*,*E*)-*N*-((4-(((*R*)-6-((*tert*-Butoxycarbonyl)amino)-2-((*R*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanamido)hexanamido)methyl)benzyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound J-1).

The title compound was prepared from Compound I-2 and Fmoc-Phe-Lys(Boc)-OH according to General Procedure 4. $C_{75}H_{100}N_8O_{13}S$ calcd. *m/z* = 1352.71 amu; found $[M+H]^+$ = 1353.96, $[M+Na]^+$ = 1375.83, $[M\text{-}Boc+2H]^+$ = 1253.78, $[M\text{-}2Boc+H]^+$ = 1153.70.

Step 2: (*S*,*E*)-*N*-((4-(((*R*)-6-((*tert*-Butoxycarbonyl)amino)-2-((*R*)-2-amino-3-phenylpropanamido)hexanamido)methyl)benzyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound J-2).

The title compound was prepared from Compound J-1 according to General Procedure 5. $C_{60}H_{90}N_8O_{11}S$ calcd. *m/z* = 1130.64 amu; found $[M+H]^+$ = 1131.75, $[M+Na]^+$ = 1153.75, $[M\text{-}Boc+2H]^+$ = 1031.68, $[M\text{-}2Boc+3H]^+$ = 931.61.

Step 3: (*S*,*E*)-*N*-(4-(((*R*)-6-((*tert*-Butoxycarbonyl)amino)-2-((*R*)-2-(6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-phenylpropanamido)hexanamido)methyl)benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound J-3).

The title compound was prepared from Compound J-2 and MC-NHS according to General Procedure 6. $C_{70}H_{101}N_9O_{14}S$ calcd. *m/z* = 1323.72 amu; found $[M+H]^+$ = 1324.96, $[M+Na]^+$ = 1346.94, $[M\text{-}Boc+2H]^+$ = 1224.87, $[M\text{-}2Boc+3H]^+$ = 1124.79.

Step 4: (*S*,*E*)-*N*-(4-(((*R*)-6-Amino-2-((*R*)-2-(6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-phenylpropanamido)hexanamido)methyl)benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound J).

The title compound was prepared from Compound J-3 according to General Procedure 7. $C_{60}H_{85}N_9O_{10}S$ calcd. *m/z* = 1123.61 amu; found $[M+H]^+$ = 1124.75, $[M+Na]^+$ = 1146.77, $[(M+2H)/2]^{2+}$ = 563.09.

Example 2.18: Preparation of (*S*,*E*)-*N*-(4-((*R*)-2-((*R*)-2-(6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound K).

Step 1: 2,2,2-Trifluoro-*N*-(4-(Sulfamoylmethylphenyl)acetamide.

The title compound was synthesized from commercially available (4-aminophenyl)methanesulfonamide and TFAA using General Procedure 1. ^1H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H), 7.79-7.51 (m, 2H), 7.51-7.23 (m, 2H), 6.85 (s, 2H), 4.27 (s, 2H).

Step 2: (*S*,*E*)-2,5-Dimethyl-*N*-((4-(2,2,2-trifluoroacetamido)benzyl)sulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound K-1).

The title compound was synthesized from Boc-HTI-286-OH and 2,2,2-trifluoro-*N*-(4-(sulfamoylmethyl)phenyl)acetamide according to General Procedure 2. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.81 (s, 1H), 7.66-7.50 (m, 3H), 7.50-7.31 (m, 5H), 7.23 (t, *J* = 7.7 Hz, 1H), 6.35 (dd, *J* = 9.2, 1.6 Hz, 1H), 6.22 (d, *J* = 8.8 Hz, 1H), 5.34 (s, 1H), 5.05-4.80 (m, 3H), 4.72-4.40 (m, 2H), 2.97-2.74 (m, 3H), 2.60 (s, 3H), 1.95 (m, 4H), 1.68-1.35 (m, 15H), 1.02-0.63 (m, 15H). $C_{41}H_{58}F_3N_5O_8S$ calcd. $[M+H]^+$ 838.40; found $[M+Na]^+$ 860.48; $[M+H]^+$ 838.52; $[M\text{-Boc}+2H]^+$ 738.39.

Step 3: (*S*,*E*)-N-((4-Aminobenzyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound K-2).

The title compound was prepared from Compound K-2 according to General Procedure 3. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.63-7.39 (m, 2H), 7.35 (t, *J* = 7.7 Hz, 2H), 7.22 (t, *J* = 7.3 Hz, 1H), 7.16-7.03 (m, 2H), 6.73-6.54 (m, 2H), 6.36 (dd, *J* = 9.2, 1.6 Hz, 1H), 6.07 (s, 1H), 5.00 (m, 2H), 4.60 (s, 3H), 2.98-2.75 (m, 6H), 1.97-1.71 (m, 4H), 1.68-1.34 (m, 15H), 0.97-0.63 (m, 15H). $C_{39}H_{59}N_5O_7S$ calcd. $[M+H]^+$ 742.41; found $[M+H]^+$ 742.47; $[M\text{-Boc}+2H]^+$ 642.40.

Step 4: (*S*,*E*)-*N*-((4-((*R*)-2-((*R*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)benzyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound K-3).

The title compound was prepared from Compound K-2 and Fmoc-Val-Cit-OH according to General Procedure 4. $C_{65}H_{89}N_9O_{12}S$ calcd. $[M+H]^+$ 1220.64; found $[M+H]^+$ 1 220.97; $[M\text{-Boc}+2H]^+$ 1120.87.

Step 5: (*S*,*E*)-*N*-((4-((*R*)-2-((*R*)-2-Amino-3-methylbutanamido)-5-ureidopentanamido)benzyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound K-4).

The title compound was prepared from Compound K-3 according to General Procedure 5. $C_{50}H_{79}N_9O_{10}S$ calcd. $[M+Na]^+$ 998.57; found $[M+14]^+$ 998.75; $[M\text{-Boc}+H]^+$ 898.69.

Step 6: (*S*,*E*)-*N*-(4-((*R*)-2-((*R*)-2-(6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound K-5).

The title compound was prepared by reaction of Compound K-4 with MC-NHS according to General Procedure 6. $C_{60}H_{90}N_{10}O_{13}S$ calcd. $[M+H]^+$ 1191.64; found $[M+H]^+$ 1191.74; $[M\text{-}Boc+2H]^+$ 1091.67.

Step 7: (*S*,*E*)-*N*-(4-((*R*)-2-((*R*)-2-(6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

The title compound was prepared from Compound K-5 according to General Procedure 7. $C_{55}H_{82}N_{10}O_{11}S$ calcd. $[M+H]^+$ 1091.59; found $[M+H]^+$ 1091.67.

Example 2.19: Preparation of (*S*,*E*)-*N*-((4-((14*R*,17*R*)-1-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)benzyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound L).

Step 1: (*S*,*E*)-*N*-((4-((14*R*,17*R*)-1-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)benzyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound L-1).

To a stirred solution of Compound K-4 (40.0 mg, 0.040 mmol, 1.0 eq) in CH_2Cl_2 (0.5 mL) was added MT-OH (18.1 mg, 0.060 mmol, 1.5 eq). Next, triethylamine (0.017 mL, 0.120 mmol, 3.0 eq) then Mukiyama's reagent (15.4 mg, 0.060 mmol, 1.5 eq) were added. After 3 h, approximately one equivalent of acid, triethylamine, and Mukiyama's reagent was added, and after 30 more min, HPLC indicated consumption of starting material Compound K-4. The reaction mixture was diluted with 0.25 mL hexanes and loaded directly onto flash chromatography to yield the title compound (29.3 mg, 57%) as a clear yellow film. $C_{63}H_{96}N_{10}O_{16}S$ calcd. *m/z* = 1280.67. Found $[M+H]^+$ = 1281.94, $[M+Na]^+$ = 1303.91, $[M\text{-}Boc+2H]^+$ = 1181.86. R_f = 0.45 (10% (5% AcOH/MeOH)/10% Hex/CH_2Cl_2)

Step 2: (*S*,*E*)-*N*-((4-((14*R*,17*R*)-1-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)benzyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

The title compound was prepared according to General Procedure 7 from Compound L-1. $C_{58}H_{88}N_{10}O_{14}S$ calcd. *m/z* for = 1180.62. Found $[M+H]^+$ = 1181.82, $[(M+2H)/2]^{2+}$ = 591.60.

Example 2.20: (*S*,*E*)-*N*-(4-((*R*)-6-Amino-2-((*R*)-2-(6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-phenylpropanamido)hexanamido)benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-

trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound M).

Step 1: (*S*,*E*)-*N*-((4-((*R*)-6-((*tert*-Butoxycarbonyl)(methyl)amino)-2-((*R*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanamido)hexanamido)benzyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound M-1).

The title compound was prepared from Compound K-2 and Fmoc-Phe-Lys(Boc)-OH according to General Procedure 4. $C_{74}H_{98}N_8O_{13}S$ calcd. *m/z* = 1338.70 amu; found $[M+H]^+$ = 1339.96, $[M+Na]^+$ = 1361.92, $[M-Boc+2H]^+$ = 1239.85, $[M-2Boc+H]^+$ = 1139.77.

Step 2: (*S*,*E*)-*N*-((4-((*R*)-6-((*tert*-Butoxycarbonyl)(methyl)amino)-2-((*R*)-2-amino-3-phenylpropanamido)hexanamido)benzyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound M-2).

The title compound was prepared from Compound M-1 according to General Procedure 5. $C_{59}H_{88}N_8O_{11}S$ calcd. *m/z* = 1116.63 amu; found $[M+H]^+$ = 1117.78, $[M+Na]^+$ = 1139.80, $[M-Boc+H]^+$ = 1017.72, $[M-2Boc+3H]^+$ = 917.64.

Step 3: (*S*,*E*)-*N*-(4-((*R*)-6-((*tert*-Butoxycarbonyl)(methyl)amino)-2-((*R*)-2-(6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-phenylpropanamido)hexanamido)benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound M-3).

The title compound was prepared from Compound M-2 and MC-NHS according to General Procedure 6. $C_{69}H_{99}N_9O_{14}S$ calcd. *m/z* = 1309.70 amu; found $[M+H]^+$ = 1310.93, $[M+Na]^+$ = 1332.89, $[M-Boc+2H]^+$ = 1210.84, $[M-2Boc+3H]^+$ = 1110.76.

Step 4: (*S*,*E*)-*N*-(4-((*R*)-6-Amino-2-((*R*)-2-(6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-phenylpropanamido)hexanamido)benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

The title compound was prepared from Compound M-3 according to General Procedure 7. $C_{59}H_{83}N_9O_{10}S$ calcd. *m/z* = 1109.60 amu; found $[M+H]^+$ = 1110.71, $[M+Na]^+$ = 1132.74, $[(M+2H)/2]^{2+}$ = 556.18.

Example 2.21: Preparation of (*S*,*E*)-*N*-(4-((*S*)-2-((*S*)-2-(6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound N).

Step 1: 2,2,2-Trifluoro-*N*-(4-sulfamoylphenyl)acetamide.

The title compound was synthesized from commercially available sulfanilamide and TFAA using General Procedure 1.

Step 2: *tert*-Butyl (*S*)-1-((*S*)-1-(((*S,E*)-2,5-Dimethyl-6-oxo-6-(4-(2,2,2-trifluoroacetamido)phenylsulfonamido)hex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate.

The title compound was synthesized from Boc-HTI-286-OH and 2,2,2-trifluoro-*N*-(4-sulfamoylphenyl)acetamide according to General Procedure 2. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.14-8.03 (m, 2H), 7.98-7.83 (m, 3H), 7.47 (d, J = 7.6 Hz, 21-I), 7.32 (d, J = 7.6, 2H), 7.20 (q, J = 7.4, 6.2 Hz, 2H), 6.44 (d, J = 9.1 Hz, 1H), 5.16 (s, 1H), 4.68 (d, J = 9.0 Hz, I H), 3.08-2.95 (m, 3H), 2.87 (d, J = 6.4 Hz, 3H), 2.01 (m, 6H), 1.80 (d, J = 11.7 Hz, 3H), 1.62 (d, J = 6.4 Hz, 114), 1.52-1.36 (m, 14H), 1.26 (m, 1H), 0.98-0.72 (m, 15H). $C_{40}H_{56}F_3N_5O_8S$ calcd. $[M+H]^+$ 824.38; found $[M+Na]^+$ 846.43; $[M+H]^+$ 824.40; $[M\text{-}Boc+2H]^+$ 724.34.

Step 3: *tert*-Butyl (*S*)-1-((*S*)-1-(((*S,E*)-6-(4-Aminophenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate (Compound N-1c).

The title compound was prepared from *tert*-butyl (*S*)-1-((*S*)-1-(((*S,E*)-2,5-dimethyl-6-oxo-6-(4-(2,2,2-trifluoroacetamido)phenylsulfonamido)hex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate according to General Procedure 3.

Step 4: *tert*-Butyl ((*S*)-1-(((*S*)-1-(((*S,E*)-6-(4-((*S*)-2-((*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxo-3-phenylbutan-2-yl)(methyl)carbamate (Compound N-1).

Synthesized from *tert*-butyl (*S*)-1-((*S*)-1-(((*S,E*)-6-(4-aminophenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate and Fmoc-Val-Cit-OH according to General Procedure 4. $C_{64}H_{87}N_9O_{12}S$ calcd. $[M+H]^+$ 1206.62; found $[M+Na]^+$ 1230.81; $[M+H]^+$ 1206.73; $[M\text{-}Boc+2H]^+$ 1106.63.

Step 5: *tert*-Butyl (*S*)-1-((*S*)-1-(((*S,E*)-6-(4-((*S*)-2-((*S*)-2-Amino-3-methylbutanamido)-5-ureidopentanamido)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate.

The title compound was prepared from Compound N-1 according to General Procedure 5. $C_{49}H_{77}N_9O_{10}S$ calcd. $[M+H]^+$ 984.55; found $[M+H]^+$ 984.63; $[M\text{-}Boc+2H]^+$ 884.57.

Step 6: *tert*-Butyl (*S*)-1-((*S*)-1-(((*S,E*)-6-(4-((*S*)-2-((*S*)-2-(6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)phenylsulfonamido)-2,5-

dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate.

The title compound was prepared from *tert*-butyl (*S*)-1-((*S*)-1-(((*S*,*E*)-6-(4-((*S*)-2-((*S*)-2-amino-3-methylbutanamido)-5-ureidopentanamido)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate and MC-NHS according to General Procedure 6. $C_{59}H_{88}N_{10}O_{13}S$ calcd. $[M+H]^+$ 1177.63; found $[M+Na]^+$ 1199.74; $[M+H]^+$ 1177.85; $[M\text{-Boc}+2H]^+$ 1077.68.

Step 7: (*S*,*E*)-*N*-(4-((*S*)-2-((*S*)-2-(6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

The title compound was prepared from *tert*-butyl (*S*)-1-((*S*)-1-(((*S*,*E*)-6-(4-((*S*)-2-((*S*)-2-(6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate according to General Procedure 7. $C_{54}H_{80}N_{10}O_{11}S$ calcd. $[M+H]^+$ 1077.63; found $[M+H]^+$ 1077.68.

Example 2.22: Preparation of (*S*,*E*)-*N*-((4-((14*R*,17*R*)-1-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)phenyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound O).

Step 1: *tert*-Butyl ((*S*)-1-(((*S*)-1-(((*S*,*E*)-6-(4-((14*R*,17*R*)-1-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxo-3-phenylbutan-2-yl)(methyl)carbamate.

The title compound was prepared according to General Procedure 6 from *tert*-butyl (*S*)-1-((*S*)-1-(((*S*,*E*)-6-(4-((*S*)-2-((*S*)-2-amino-3-methylbutanamido)-5-ureidopentanamido)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-di methyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate and MT-NHS. *m/z* calcd. for $C_{62}H_{94}N_{10}O_{16}S$ = 1266.66. Found $[M+H]^+$ = 1267.87 $[M+Na]^+$ = 1289.86, $[M\text{-Boc}+2H]^+$ = 1167.82. R_f = 0.49 (10% (5% AcOH/MeOH)/CH_2C1_2).

Step 2: (*S*,*E*)-*N*-((4-((14*R*,17*R*)-1-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)phenyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

The title compound was prepared according to General Procedure 7 from *tert*-butyl ((*S*)-1-(((*S*)-1-(((*S*,*E*)-6-(4-((14*R*,17*R*)-1-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-

17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxo-3-phenylbutan-2-yl)(methyl)carbamate. *m/z* calcd. for $C_{57}H_{86}N_{10}O_{14}S$ = 1166.60. Found $[M+H]^+$ = 1167.67, $[(M+2H)/2]^{2+}$ = 584.57.

Example 2.23: Preparation of (*S*,*E*)-*N*-(4-(1-((*R*)-2-((*R*)-2-(6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)cyclopropyl)benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound P).

Step 1:4-(Tritylthiomethyl)benzonitrile.

Tritylmercaptan (1.48 g, 5.36 mmol, 1.05 eq) in THF (5 mL) was added dropwise to a stirred suspension of sodium hydride (60% dispersion in mineral oil, 214 mg, 5.36 mmol, 1.05 eq) in THF (5 mL) under N_2 at 0 °C. After 15 min, 4-(bromomethyl)benzonitrile (1.00 g, 5.10 mmol, 1.0 eq) in THF (5 mL) was added and the reaction was allowed to come to rt. After 1 h, TLC indicated complete conversion of starting material. The reaction was quenched by adding saturated ammonium chloride, then some dH_2O. The mixture was extracted three times with ether, washed with saturated brine, dried over sodium sulfate, and concentrated to a viscous yellow oil. Purification by flash chromatography gave the title compound (1.76 g, 88%) as a light white powder. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.52 (d, *J* = 8.2 Hz, 2H), 7.47 (d, *J* = 7.1 Hz, 6H), 7.33 (t, *J* = 7.5 Hz, 6H), 7.26 (t, *J* = 7.2 Hz, 3H), 7.19 (d, *J* = 8.2 Hz, 2H), 3.40 (s, 2H). *m/z* calcd. for $C_{27}H_{21}NS$ = 391.14. Found $[M+Na]^+$ = 414.13. R_f = 0.32 (10% EtOAc/Hex).

Step 2: 1-(4-(Tritylthiomethyl)phenyl)cyclopropanamine.

4-(Tritylthiomethyl)benzonitrile (1.47 g, 3.75 mmol, 1.0 eq) was taken up in 40 mL THF, under N_2 atmosphere, then cooled to -78 °C. To this solution was added Ti(O-*i*Pr)$_4$ (1.21 mL, 4.13 mmol, 1.1 eq), then ethylmagnesium bromide (3 M, 2.75 mL, 8.26 mmol, 2.2 eq) was added dropwise over 5 min. The dry-ice bath was removed, allowing the solution to reach rt. After 45 min at rt, BF_3•Et_2O (0.93 mL, 7.51 mmol, 2.0 eq) was added to the now very dark reaction mixture. After stirring for an additional 2.5 h, the reaction was quenched with 5 mL of 2 M HCl, followed by pH adjustment to strong base with about 15 mL 2 M NaOH. Some water was added to the mixture, then it was extracted three times with 75 mL EtOAc, washed once with dH_2O, once with saturated brine, dried over sodium sulfate, and concentrated to a clear oil. The material was purified by flash chromatography to afford the title compound (680 mg, 36%) as a clear oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.49 (d, *J* = 7.8 Hz, 6H), 7.33 (t, *J* = 7.7 Hz, 6H), 7.26 (t, *J* = 7.2 Hz, 3H), 7.20 (d, *J* = 8.2 Hz, 2H), 7.11 (d, *J* = 8.2 Hz, 2H), 3.32 (s, 2H), 1.06 (dd, *J* = 7.9, 5.0 Hz, 2H), 0.95 (dd, *J* = 7.9, 4.7 Hz, 2H). *m/z* calcd. for $C_{29}H_{27}NS$ = 421.19. Found $[M+H]^+$ = 422.19. R_f = 0.21 (50% EtOAc/Hex).

Step 3: 2,2,2-Trifluoro-*N*-(1-(4-(tritylthiomethyl)phenyl)cyclopropyl)acetamide.

To a stirred solution of 1-(4-(tritylthiomethyl)phenyl)cyclopropanamine (680 mg, 1.61 mmol, 1.0 eq) in CH_2Cl_2 was added trifluoroacetic anhydride (0.448 mL, 3.22 mmol, 2.0 eq) and triethylamine (0.45 mL, 3.22 mmol, 2.0 eq). After two hours, TLC and HPLC indicated complete conversion of starting material. The reaction was quenched by the addition of 3 mL $NaHCO_3$, then some dH_2O was added, and the mixture was extracted three times with CH_2C1_2. The combined organics were washed with saturated brine, dried over sodium sulfate, and concentrated to a yellow foam, giving the title compound (715 mg, 86%) in sufficient purity to move to the next step. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.48 (d, *J* = 7.7 Hz, 6H), 7.32 (t, *J*= 7.6 Hz, 6H), 7.25 (t, *J* = 7.2 Hz, 3H), 7.19 (d, *J* = 8.2 Hz, 2H), 7.10 (d, *J*= 8.3 Hz, 2H), 6.83 (s, 1H), 3.31 (s, 2H), 1.40-1.24 (m, 4H). *m/z* calcd. for $C_{31}H_{26}F_3NOS$ = 517.17. Found $[M+Na]^+$ = 540.25. R_f = 0.71 (50% EtOAc/Hex).

Step 4: 2,2,2-Trifluoro-*N*-(1-(4-(mercaptomethyl)phenyl)cyclopropyl)acetamide.

2,2,2-Trifluoro-*N*-(1-(4-(tritylthiomethyl)phenyl)cyclopropyl)acetamide (715 mg, 1.38 mmol, 1.0 eq) in 5 mL CH_2C1_2 was treated with 2.5 mL TFA. After 1 min, TIPSH (0.42 mL, 2.1 mmol, 1.5 eq) was added, causing the yellow color to fade. After 30 min, TLC indicated the reaction to be complete. The mixture was concentrated, then co-evaporated once with CH2C12 and twice with toluene. The residue was purified by flash chromatography to afford the title compound (261 mg, 69%) as a white solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.35 -7.23 (m, 4H), 6.87 (s, 1H), 3.74 (d, *J* = 7.6 Hz, 2H), 1.77 (t, *J* = 7.6 Hz, 1H), 1.36 (s, 4H). R_f = 0.47 (20% EtOAc/Hex).

Step 5: 2,2,2-Trifluoro-*N*-(1-(4-(sulfamoylmethyl)phenyl)cyclopropyl)acetamide.

To a stirred solution of 2,2,2-trifluoro-*N*-(1-(4-(mercaptomethyl)phenyl)cyclopropyl)acetamide (220 mg, 0.799 mmol, 1.0 eq) in acetonitrile were added dH_2O (0.029 mL, 1.6 mmol, 2.0 eq), tetrabutylammonium chloride (110 mg, 0.40 mmol, 0.5 eq), then *N*-chlorosuccinimide (320 mg, 2.40 mmol, 3.0 eq). After 20 minutes, no starting material was visible by TLC. After 90 min, concentrated NH_4OH (0.18 mL, 3.2 mmol, 4.0 eq) was added. After 10 minutes, 1 mL of NH_4C1 was added, and the mixture was extracted three times with EtOAc. The combined organics were washed twice with dH_2O, once with saturated brine, dried over sodium sulfate, and concentrated to a clear oil. The residue was purified by flash chromatography to afford the title compound (192 mg, 74%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.21 (s, 1H), 7.31 (d, *J* = 8.2 Hz, 2H), 7.16 (d, *J* = 8.3 Hz, 2H), 6.85 (s, 2H), 4.23 (s, 2H), 1.27 (dt, *J* = 6.1, 2.3 Hz, 4H). R_f = 0.26 (50% EtOAc/Hex).

Step 6: (*S*,*E*)-2,5-Dimethyl-*N*-((4-(1-(2,2,2-trifluoroacetamido)cyclopropyl)benzyl)sulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound P-1).

The title compound was prepared according to General Procedure 2 from 2,2,2-trifluoro-*N*-(1-(4-(sulfamoylmethyl)phenyl)cyclopropyl)acetamide and Boc-HTI-286-OH. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.54 (s, 1H), 7.78 (s, 1H), 7.36 (d, *J* = 7.1 Hz, 2H), 7.31-7.23 (m, 2H), 7.23-7.11 (m, 5H), 6.33 (d, *J* = 9.3 Hz, 1H), 6.28-6.14 (m, 1H), 5.35 (s, 1H), 4.97 (t, *J* = 10.3 Hz, 1H), 4.84 (d, *J* = 13.7 Hz, 1H), 4.70-4.56 (m, 1H), 4.50 (d, = 8.9 Hz, 1H), 2.90 (s, 3H), 2.59 (s, 3H), 1.90 (s, 3H), 1.82-1.72 (m, 1H), 1.62-1.57 (m, 3H), 1.55 (s, 3H), 1.47 (s, 9H), 1.45-1.34 (m, 4H), 0.85 (d, *J* = 6.5 Hz, 2H), 0.82-0.67 (m, 12H). *m/z* calcd. for $C_{44}H_{62}F_3N_5O_8S$ = 877.43. Found $[M+Na]^+$ = 900.67. R_f = 0.34 (50% (2% AcOH/EtOAc)/Hex).

Step 7: (*S*,*E*)-*N*-((4-(1-Aminocyclopropyl)benzyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound P-2).

The title compound was prepared according to General Procedure 3 in MeOH/H_2O from Compound P-1. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.62 -7.48 (m, 4H), 7.35 (t, *J* = 7.6 Hz, 2H), 7.31-7.12 (m, 3H), 6.51 (d, *J* = 6.8 Hz, 1H), 6.36-6.18 (m, 1H), 5.29 (s, 1H), 5.00-4.86 (m, 1H), 4.67 (s, 2H), 4.60 (d, *J* = 9.3 Hz, 1H), 3.07-2.73 (m, 6H), 2.02-1.84 (m, 4H), 1.68-1.51 (m, 6H), 1.47 (s, 9H), 1.45-1.38 (m, 2H), 1.16 (s, 2H), 0.89-0.81 (m, 1211), 0.80 (d, *J* = 6.7 Hz, 3H). *m/z* calcd. for $C_{42}H_{63}N_5O_7S$ = 781.44. Found $[M+H]^+$ = 782.63.

Step 8: (*S*,*E*)-*N*-((4-(1-((*R*)-2-((*R*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)cyclopropyl)benzyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound P-3).

The title compound was prepared according to General Procedure 4 from Compound P-2 and Fmoc-Val-Cit-OH. *m/z* calcd. for $C_{68}H_{93}N_9O_{12}S$ = 1259.67. Found $[M+H]^+$ = 1261.11, $[M+Na]^+$ = 1283.06, $[M-Boc+2H]^+$ = 1160.97. R_f = 0.54 (5% MeOH/(2% AcOH/EtOAc)).

Step 9: (*S*,*E*)-*N*-((4-(1-((*R*)-2-((*R*)-2-Amino-3-methylbutanamido)-5-ureidopentanamido)cyclopropyl)benzyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound P-4).

The title compound was prepared according to General Procedure 5 from Compound P-3. *m/z* calcd. for $C_{53}H_{83}N_9O_{10}S$ = 1037.60. Found $[M+H]^+$ = 1038.90, $[M-Boc+2H]^+$ = 938.78. R_f ~ 0.1 (25% MeOH/CH_2C1_2).

Step 10: (*S*,*E*)-*N*-(4-(1-((*R*)-2-((*R*)-2-(6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)cyclopropyl)benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound P-5).

The title compound was prepared according to General Procedure 6 from Compound P-4 and MC-NHS. *m/z* calcd. for $C_{63}H_{94}N_{10}O_{13}S$ = 1230.67. Found $[M+H]^+$ =1232.11, $[M+Na]^+$ = 1254.09, $[M-Boc+2H]^+$ = 1132.01. R_f= 0.44 (10% (5% AcOH/MeOH)/CH_2C1_2).

Step 11: (*S*,*E*)-*N*-(4-(1-((*R*)-2-((*R*)-2-(6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)cyclopropyl)benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

The title compound was prepared according to General Procedure 7 from Compound P-5. *m/z* calcd. for $C_{58}H_{86}N_{10}O_{11}S$ = 1130.62. Found $[M+H]^+$ =1131.95, $[(M+2H)/2]^{2+}$ = 566.69.

Example 2.24: (*S*,*E*)-*N*-(4-(1-((*R*)-2-((*R*)-2-(6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)cyclopropyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound Q).

Step 1: 1-Phenylcyclopropanamine.

The title compound was prepared as described in Bertus, P., Szymoniak, J. J. Org. Chem., 2003, 68, 7133-7136 from benzonitrile (1.0 mL, 9.7 mmol) to give 270 mg (21%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.44-7.28 (m, 4H), 7.27-7.15 (m, 1H), 1.18-1.06 (m, 2H), 1.07-0.95 (m, 2H). R_f = 0.28 (5% (5% NH_4OH/MeOH)/CH_2C1_2).

Step 2: 2,2,2-Trifluoro-*N*-(1-phenylcyclopropyl)acetamide.

To a stirred solution of 1-phenylcyclopropanamine (270 mg, 2.03 mmol, 1.0 eq) in dioxane (5 mL), was added trifluoroacetic anhydride (0.310 mL, 2.23 mmol, 1.1 eq). After 5 min, TLC indicated complete conversion of starting material. The mixture was concentrated, then coevaporated once with CH_2C1_2 and once with toluene to yield the title compound (453 mg, 97%) as a flaky white powder. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.47-7.15 (m, 5H), 6.88 (s, 1H), 1.65 (s, 4H). *m/z* calcd. for $C_{11}H_{10}F_3NO$ = 229.07. Found $[M+H]^+$ = 230.14. R_f = 0.82 (5% (5% NH_4OH/MeOH)/CH_2C1_2).

Step 3: 2,2,2-Trifluoro-*N*-(1-(4-sulfamoylphenyl)cyclopropyl)acetamide.

To stirred chlorosulfonic acid (0.78 mL, 11.8 mmol, 6.0 eq) at 0 °C, was added solid 2,2,2-trifluoro-*N*-(1-phenylcyclopropyl)acetamide (450 mg, 1.96 mmol, 1.0 eq) portionwise, keeping the temperature low. After complete addition, the mixture was heated to 50 °C. After 1 minute, gas evolution ceased, and the reaction was allowed to cool. The mixture was added slowly to a beaker of ice, being mindful of splattering. The solid that was left in the ice was filtered off. This solid was dried *in vacuo* and then taken up in THF (4 mL). Concentrated NH_4OH (0.44 mL, 7.85 mmol, 4.0 eq) was added, turning the solution green-black. After 2 min, TLC indicated complete consumption of the sulfonylchloride intermediate. 2M HCl was added until the color faded, then the mixture was extracted three times with EtOAc, washed once with saturated $NaHCO_3$, once with saturated brine,

dried over sodium sulfate, and concentrated to a flaky solid. The crude material was purified by flash chromatography to yield the title compound (235 mg, 39%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.28 (s, 1H), 7.76 (d, J = 8.5 Hz, 2H), 7.32 (d, J = 8.1 Hz, 2H), 7.31 (s, 2H), 1.42-1.35 (m, 2H), 1.35-1.27 (m, 2H). *m/z* calcd. for $C_{11}H_{11}F_3N_2O_3S$ = 308.04. Found $[M+H]^+$ = 309.07. R_f = 0.27 (50% EtOAc/Hex).

Step 4: *tert*-Butyl (*S*)-1-((*S*)-1-(((*S,E*)-2,5-Dimethyl-6-oxo-6-(4-(1-(2,2,2-trifluoroacetamido)cyclopropyl)phenylsulfonamido)hex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate.

The title compound was prepared according to General Procedure 2 from 2,2,2-trifluoro-*N*-(1-(4-sulfamoylphenyl)cyclopropyl)acetamide and Boc-HTI-286-OH. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.51 (s, 1H), 8.08 (d, J = 8.6 Hz, 2H), 7.42-7.32 (m, 2H), 7.32-7.23 (m, 2H), 7.23-7.10 (m, 3H), 6.46 (d, J = 9.0 Hz, 1H), 6.17-6.08 (m, 1H), 5.29 (s, 1H), 4.97-4.76 (m, 1H), 4.56 (d, J = 8.8 Hz, 1H), 2.90 (d, J = 10.4 Hz, 6H), 2.01-1.79 (m, 4H), 1.62 (s, 3H), 1.53 (s, 3H), 1.49 (s, 4H), 1.46 (s, 9H), 0.86 (t, J = 6.9 Hz, 3H), 0.81 (d, = 6.8 Hz, 3H), 0.77 (s, 9H). *m/z* calcd. for $C_{43}H_{60}F_3N_5O_8S$ = 863.41. Found $[M+H]^+$ = 864.56, $[M+Na]^+$ = 886.52, $[M-Boc+2H]^+$ = 764.44. R_f = 0.34 (50% (2% AcOH/EtOAc)/Hex).

Step 5: *tert*-Butyl (*S*)-1-((*S*)-1-(((*S,E*)-6-(4-(1-Aminocyclopropyl)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate.

The title compound was prepared according to General Procedure 3 in dioxanes from compound *tert*-butyl (*S*)-1-((*S*)-1-(((*S,E*)-2,5-dimethyl-6-oxo-6-(4-(1-(2,2,2-trifluoroacetamido)cyclopropyl)phenylsulfonamido)hex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate. ^{1}H NMR (400 MHz, Methanol-*d*4) δ 7.97 (d, J = 8.5 Hz, 2H), 7.52 (d, J = 8.5 Hz, 2H), 7.51-7.43 (m, 2H), 7.32 (t, J = 7.5 Hz, 2H), 7.20 (t, J = 8.4 Hz, 1H), 6.55 (d, J = 9.0 Hz, 1H), 5.17 (s, 1H), 5.03-4.94 (m, 1H), 4.70 (d, J = 9.0 Hz, 1H), 2.94 (s, 3H), 2.88 (s, 3H), 1.94-1.89 (m, 1H), 1.80 (s, 3H), 1.53 (s, 3H), 1.51 (s, 3H), 1.43 (s, 9H), 1.40-1.37 (m, 2H), 1.36-1.32 (m, 2H), 0.87 (d, J = 6.0 Hz, 12H), 0.82-0.76 (m, 3H). *m/z* calcd. for $C_{41}H_{61}N_5O_7S$ = 767.43. Found $[M+H]^+$ = 768.51 $[M-Boc+2H]^+$ = 668.38. R_f = 0.32 (10% EtOAc/Hex).

Step 6: *tert*-Butyl ((*S*)-1-(((*S*)-1-(((*S,E*)-6-(4-(1-((*R*)-2-((*R*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)cyclopropyl)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxo-3-phenylbutan-2-yl)(methyl)carbamate (Compound Q-1).

The title compound was prepared according to General Procedure 4 from *tert*-butyl (*S*)-1-((*S*)-1-(((*S,E*)-6-(4-(1-aminocyclopropyl)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-

yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate and Fmoc-Val-Cit-OH. *m/z* calcd. for $C_{67}H_{91}N_9O_{12}S$ = 1245.65. Found $[M+H]^+$ = 1246.89, $[M+Na]^+$ = 1268.88, $[M\text{-}Boc+2H]^+$ = 1146.82. R_f = 0.52 (5% MeOH/(2%AcOH/EtOAc)).

Step 7: *tert*-Butyl (*S*)-1-((*S*)-1-(((*S,E*)-6-(4-(1-((*R*)-2-((*R*)-2-Amino-3-methylbutanamido)-5-ureidopentanamido)cyclopropyl)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate (Compound Q-2).

The title compound was prepared according to General Procedure 5 from Compound Q-1. *m/z* calcd. for $C_{52}H_{81}N_9O_{10}S$ = 1023.58. Found $[M+H]^+$ = 1024.72, $[M\text{-}Boc+2H]^+$ = 924.66.

Step 8: *tert*-Butyl (*S*)-1-((*S*)-1-(((*S,E*)-6-(4-(1-((*R*)-2-((*R*)-2-(6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)cyclopropyl)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate.

The title compound was prepared according to General Procedure 6 from *tert*-butyl (*S*)-1-((*S*)-1-(((*S,E*)-6-(4-(1-((*R*)-2-((*R*)-2-amino-3-methylbutanamido)-5-ureidopentanamido)cyclopropyl)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate and MC-NHS. *m/z* calcd. for $C_{62}H_{92}N_{10}O_{13}S$ = 1216.66. Found $[M+H]^+$ = 1217.89, [M+Na]+ = 1239.94, $[M\text{-}Boc+2H]^+$ = 1117.82. R_f = 0.39 (10% (5% AcOH/MeOH)/CH_2C_{12}).

Step 9: (*S,E*)-*N*-(4-(1-((*R*)-2-((*R*)-2-(6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)cyclopropyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

The title compound was prepared according to General Procedure 7 from compound *tert*-butyl (*S*)-1-((*S*)-1-(((*S,E*)-6-(4-(1-((*R*)-2-((*R*)-2-(6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)cyclopropyl)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate. *m/z* calcd. for $C_{57}H_{84}N_{10}O_{11}S$ = 1116.60. Found $[M+H]^+$ = 1117.77, $[(M+2H)/2]^{2+}$ = 559.56.

Example 2.25: 2,5-Dioxopyrrolidin-1-yl 6-((*R*)-1-((*R*)-1-(4-(*N*-((*S,E*)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enoyl)sulfamoyl)phenylamino)-1-oxo-5-ureidopentan-2-ylamino)-3-methyl-1-oxobutan-2-ylamino)-6-oxohexanoate (Compound KK).

Step 1: 2,2,2-Trifluoro-*N*-(4-sulfamoylphenyl)acetamide.

The title compound was synthesized from commercially available sulfanilamide and TFAA using General Procedure 1.

Step 2: *tert*-Butyl (*S*)-1-((*S*)-1-(((*S,E*)-2,5-Dimethyl-6-oxo-6-(4-(2,2,2-trifluoroacetamido)phenylsulfonamido)hex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate.

The title compound was synthesized from Boc-HTI-286-OH and 2,2,2-trifluoro-*N*-(4-sulfamoylphenyl)acetamide according to General Procedure 2. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.14 – 8.03 (m, 2H), 7.98 – 7.83 (m, 3H), 7.47 (d, J = 7.6 Hz, 2H), 7.32 (d, J = 7.6, 2H), 7.20 (q, J = 7.4, 6.2 Hz, 2H), 6.44 (d, J = 9.1 Hz, 1H), 5.16 (s, 1H), 4.68 (d, J = 9.0 Hz, 1H), 3.08 – 2.95 (m, 3H), 2.87 (d, J = 6.4 Hz, 3H), 2.01 (m, 6H), 1.80 (d, J = 11.7 Hz, 3H), 1.62 (d, J = 6.4 Hz, 1H), 1.52 – 1.36 (m, 14H), 1.26 (m, 1H), 0.98 – 0.72 (m, 15H). $C_{40}H_{56}F_3N_5O_8S$ calcd. $[M+H]^+$ 824.38; found $[M+Na]^+$ 846.43; $[M+H]^+$ 824.40; $[M\text{-}Boc+H]^+$ 724.34. MS found; 846.43 $[M+Na]^+$; 824.40 $[M+H]^+$; 724.34 $[M\text{-}Boc+H]^+$.

Step 3: *tert*-Butyl ((*S*)-1-(((*S*)-1-(((*S,E*)-6-(4-((*R*)-2-((*R*)-2-((*tert*-Butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxo-3-phenylbutan-2-yl)(methyl)carbamate.

The title compound was synthesized from *tert*-butyl (*S*)-1-((*S*)-1-(((*S,E*)-2,5-dimethyl-6-oxo-6-(4-(2,2,2-trifluoroacetamido)phenylsulfonamido)hex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-3-methyl-1-oxo-3-phenylbutan-2-yl(methyl)carbamate by first liberation of the aniline from the trifluoroacetanilide according to General Procedure 4, followed by coupling with Boc-Val-Cit-OH (synthesized according to US2010/0233190) according to General Procedure 5. A small sample was deprotected according to General Procedure 9 to resolve rotamers and facilitate NMR analysis. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.00 (d, J = 8.9 Hz, 2H), 7.83 (d, J = 8.9 Hz, 2H), 7.55 (d, J = 7.8 Hz, 2H), 7.47 (t, J = 7.7 Hz, 2H), 7.38 (t, J = 7.2 Hz, 1H), 6.51 – 6.44 (m, 1H), 5.05 – 4.97 (m, 1H), 4.64 (dd, J = 9.3, 4.7 Hz, 1H), 4.35 (s, 1H), 3.77 – 3.70 (m, 1H), 3.30 – 3.20 (m, 1H), 3.21 – 3.08 (m, 4H), 2.51 (s, 3H), 2.30-2.20 (m, 1H), 2.13 – 1.99 (m, 1H), 1.99 – 1.71 (m, 4H), 1.72 – 1.54 (m, 2H), 1.47 (s, 3H), 1.37 (s, 3H), 1.16 – 0.99 (m, 15H), 0.91 (t, J = 6.2 Hz, 6H). $C_{54}H_{85}N_9O_{12}S$ calcd. $[M+H]^+$ 1083.60; found $[M+Na]^+$ 1106.8.

Step 4: 2,5-Dioxopyrrolidin-1-yl 6-((*R*)-1-((*R*)-1-(4-(*N*-((*S,E*)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enoyl)sulfamoyl)phenylamino)-1-oxo-5-ureidopentan-2-ylamino)-3-methyl-1-oxobutan-2-ylamino)-6-oxohexanoate.

To a solution of *tert*-butyl ((*S*)-1-(((*S*)-1-(((*S,E*)-6-(4-((*R*)-2-((*R*)-2-((*tert*-butoxycarbonyl)amino)-3-methylbutanamido)-5-ureidopentanamido)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxo-

3-phenylbutan-2-yl)(methyl)carbamate (0.05 g, 0.046 mmol) in dichloromethane (2 mL) was added trifluoroacetic acid (1 mL). The reaction was monitored by HPLC-MS and upon completion, evaporated under reduced pressure and twice concentrated from toluene to remove excess TFA. The resulting residue was dissolved in *N*,*N*-dimethylformamide (2 mL). The solution was stirred, cooled to 0 °C and di-isopropylethylamine (0.008 mL, 1 equiv) and bis-*N*-Hydroxysuccinimidyl adipate (prepared according to Mishra *et al.*, Molecular Pharmaceutics, 10, (10), 3903-3912, 2013, 0.062 g, 4 equiv) were added. The reaction was allowed to stir overnight at which time HPLC-MS indicated that the starting peptide had been converted to new product. The reaction was concentrated under reduced pressure, dissolved in acetone and the resulting solution purified by prep-scale HPLC to afford the title compound (0.0145 g) as a white powder after lyophilization. ^{1}H NMR (400 MHz, DMSO-d_6 with D_2O exchange) δ 8.68 (d, J = 8.1 Hz, 1H), 7.92 – 7.83 (m, 2H), 7.80 (d, J = 8.8 Hz, 2H), 7.47 (d, J = 7.8 Hz, 2H), 7.41 (t, J = 7.6 Hz, 2H), 7.31 (dd, J = 8.3, 6.1 Hz, 1H), 6.49 (d, J = 9.1 Hz, 1H), 4.86 (t, J = 9.9 Hz, 1H), 4.75 (d, J = 8.1 Hz, 1H), 4.37 (d, J = 7.3 Hz, 2H), 4.17 (d, J = 6.9 Hz, 1H), 3.10 – 2.87 (m, 5H), 2.80 (s, 4H), 2.71 – 2.60 (m, 2H), 2.37 – 2.11 (m, 5H), 2.01-1.92 (m, 2H), 1.69 (s, 3H), 1.64 – 1.58 (m, 5H), 1.36 (s, 5H), 1.22 (s, 3H), 0.95 (s, 9H), 0.81 (m, 12H). $C_{54}H_{80}N_{10}O_{13}S$ calcd. m/z = 1108.56 found $[M+H]^+$ = 1109.54.

Example 3: Preparation of T-L^1-P^1.

Example 3.1: General Procedure 8 – Trifluoroacetamide Installation.

To a stirred suspension of the amine in 1,4-dioxane was added trifluoroacetic anhydride (1.1 equivalents). The reaction mixture transitioned from a suspension to a solution and back to a suspension again. The progress of the reaction was monitored by TLC and/or HPLC-MS for completion. Once the starting material was fully consumed, the reaction was diluted with hexanes or diethyl ether, filtered on a Buchner funnel and the resulting solids were dried under reduced pressure to give the pure trifluoroacetamide.

Example 3.2: General Procedure 9 – DCC/DMAP Mediated *N*-acyl Sulfonamide Formation.

To a stirred solution of the acid in dichloromethane was added a solution of the sulfonamide (1.3 equivalents, in dichloromethane, *N*,*N*-dimethylformamide, or a mixture thereof, as necessary). Dicyclohexylcarbodiimide (1.2 equivalents) was added and subsequently *N*,*N*-dimethylaminopyridine (1.2 equivalents). Reaction course was monitored by HPLC-MS (typically 16 h) and excess by-products could be precipitated by the addition of diethyl ether. Solids were removed by filtration and washed with 1:1 diethyl ether/dichloromethane. The combined organic layers were concentrated, and the residue was purified by silica gel chromatography or optionally prep-HPLC to give the desired *N*-acyl sulfonamide.

Example 3.3: General Procedure 10 – General Saponification.

To a solution of the trifluoroacetamide or ester containing construct in 1,4-dioxane or methanol was added lithium hydroxide (10 equivalents) and water (10% v/v). The reaction was allowed to stir at room temperature or optionally heated to 50 °C. Reaction course was monitored by HPLC-MS. Upon completion, volatiles were removed under reduced pressure, the aqueous layer was pH adjusted if necessary and washed successively with dichloromethane or ethyl acetate. The organic phases were pooled, dried over $MgSO_4$, filtered and concentrated. The reaction product was either used "as is" or purified by silica gel chromatography as necessary.

Example 3.4: General Procedure 11 – HATU mediated peptide bond formation.

To a stirred solution of the carboxylic acid in a minimal amount of dichloromethane or *N,N*-dimethylformamide or mixture thereof, at 0 °C was added HATU (equivalents) and *N,N*-diisopropylethylamine (4 equivalents). Stirring was continued for a brief induction period (5-20 minutes) at which time the reaction was charged with a solution of the amine in dichloromethane. The reaction was allowed to warm to room temperature and monitored for progress by HPLC-MS. Upon completion, volatiles were removed under reduced pressure and the residual material was purified by silica gel chromatography or reverse phase HPLC to furnish amide in adequate purity.

Example 3.5: General Procedure 12 – Boc group Removal.

To a solution of the Boc-protected construct in dichloromethane was added 10% v/v trifluoroacetic acid. Reaction course was monitored by HPLC-MS. Upon completion, all volatiles were removed under reduced pressure. The residual material was purified either by reverse phase HPLC, silica gel chromatography or precipitation from a mixture of cold methanol/dichloromethane/diethyl ether.

Example 3.6: General Procedure 13 – Pd-Catalyzed Suzuki Cross Coupling.

A suspension of aryl bromide, aryl (or alkenyl) boronic acid (1.5 eq), $Pd(OAc)_2$ (10 mol%), 2-(di-*tert*-butylphosphino)biphenyl (20 mol%), and K_3PO_4 (3 eq) in THF was stirred under N_2 at ambient temperature for 16 h (or 50 °C for 2 h). The resulting brown reaction mixture was dilute with ether and washed with 1 M NaOH (3×). The aqueous washes were combined and extracted with ether (2×). The organics were combined, dried over $MgSO_4$, filtered, concentrated *in vacuo* and purified via silica gel column chromatography (eluted with $MeOH/CH_2Cl_2$ mixtures) to afford the cross-coupled product.

Example 3.7: General Procedure 14 – Cu-Catalyzed Ullman Cross Coupling (Methoxy Installation).

A mixture of aryl bromide, CuBr (20 mol%), NaOMe (20 eq, 4.9 M in MeOH), and EtOAc (1.5 eq) was stirred under N_2 at 95 °C for 16 h. The resulting mixture was diluted with H_2O and poured into cold (0 °C) stirring 1 M citric acid. After stirring for 10 min, the mixture was extracted with EtOAc (4×). The organics were combined, washed with H_2O (2×) and brine (1×), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was used in the next step without further purification.

Example 3.8: General Procedure 15 – Vinylogous Amino Ester Synthesis.

The procedure for Weinreb amide synthesis, reduction and subsequent olefination thereof as described by Nieman J. A. *et al.* J. Nat. Prod. 2003, 66, 183-199 was employed to the desired commercially available amino acids with no modifications.

Example 3.9: General Procedure 16 – Establishment of Boc-*t*-Leucine-(Me)-Vinylogous Amino Acid.

The vinylogous amino ester was deprotected and coupled to Boc-*t*-leucine according to procedures described by Nieman J. A. *et al.* J. Nat. Prod. 2003, 66, 183-199 with no modifications.

Example 3.10: General Procedure 17 – Sulfonamide Formation from Alkyl Halide.

To a suspension of the desired alkyl halide in 2:1 H_2O/EtOH was added sodium sulfite (1.2 equiv). The resulting mixture was heated to reflux for 6-24 h. The reaction was then cooled to room temperature, the solvents were removed at reduced pressure to remove ethanol and the product was precipitated. The sodium alkylsulfonate were filtered, collected and dried *in vacuo*. These solids were then suspended in dichloromethane and phosphorous pentachloride (2 equiv) was added with stirring. The resulting suspension was heated to reflux for 2 h and allowed to cool to room temperature. The reactions were then cooled to 0 °C and water was added dropwise to consume excess phosphorous pentachloride. The mixture was transferred to a separatory funnel and the organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated to give the desired sulfonyl chloride. The thusly derived chloride was subsequently dissolved in THF and added dropwise to a stirred aqueous solution of concentrated ammonium hydroxide at 0 °C. Upon completion of the addition, the reaction was concentrated under reduced pressure and diluted with water and ethyl acetate. The organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated to give the desired sulfonamide in sufficient purity for further use.

Example 3.11: General Procedure 18 – Sulfonamide Formation from Substituted Aryl Compounds.

To a stirred mixture of the desired aryl substituted compound in chloroform was added chlorosulfonic acid (4 equiv). The reaction was heated to 70 °C for 1 h and allowed to cool to room temperature. Thionyl chloride (2 equiv) was added and the reaction was again heated to 70 °C for 1h. The contents of the reaction vessel were concentrated under reduced pressure to give an oil which was subsequently twice dissolved in toluene and concentrated under reduced pressure to remove residual acid. The remaining material was dissolved in THF and added dropwise to a concentrated, stirred solution of ammonium hydroxide at 0 °C. Once the addition was complete, the reaction was concentrated under reduced pressure and the residue was partitioned between ethyl acetate and water. The organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated to give the desired phenylsulfonamide in adequate purity for further use.

Example 3.12: General Procedure 19 – Sulfamamide Formation.

The procedures used to generate the desired sulfamamides were adapted from Winum, J.-Y. *et al.*, Org Lett, 2001, 3(14), 2241-2243

Example 3.13: General Procedure 20 – Preparation of MC-VC-PABC-Toxins (L^1-P^1)

The appropriate intermediate amine or aniline was taken up in DMF (~90 mg/mL), and to this was added 1-hydroxybenzotriazole hydrate (0.3 eq), then commercially obtained MC-VC-PABC-PNP (4-((*R*)-2-((*R*)-2-(6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate) (1.3 eq) as described in Firestone, *et al.* US6214345 was added followed by pyridine (25 eq). The reaction was covered to protect from light and stirred at ambient temperature for 24 to 48 h. The reaction mixture could be purified by concentrating the mixture and performing flash chromatography directly on the crude, or alternatively, it could be diluted with DMSO to an appropriate volume and injected directly onto a preparatory HPLC to give the pure MC-VC-PABC-R construct.

All sulfonamides and sulfamamides or precursors to the materials used in the procedures below were purchased commercially and manipulated, if necessary, such that they were suitable for use. Specifically, General Procedures 8, 17, 18 and 19 were employed to manipulate commercially available starting materials unless otherwise noted below. Sulfamamide analogs of the *N*-acyl sulfonamide containing compounds disclosed herein may be synthesized by the artisan of reasonable

skill based on the teachings herein and knowledge in the art, and are included within the scope of the invention.

Example 3.14: 3-Bromopropane-1-sulfonamide.

To a stirred slurry of potassium bromide (1.904 g) in water (2.8 mL) was added 1,3-propanesultone. The reaction was heated to 60 °C with stirring for 1h and allowed to cool to room temperature. Ethanol (~45 mL) was added with stirring and a precipitate formed. The suspension was filtered on a Buchner funnel and the solids were collected and dried at high vacuum over night to give potassium 3-bromopropane-1-sulfonate (2.90 g, 12.0 mmol) as a white solid. The above solid was added to a round bottom flask equipped with a stir bar. Phosphorous pentachloride (3.22 g, 1.3 equiv) was added in a single charge and the flask was gently shaken to mix the solids. A gas was observed to form and the solids became slightly molten. A singular drop of water was added to the mixture and a vigorous evolution of gas was observed, with more significant melting of the reaction mixture. The flask was submerged in an oil bath at 70 °C and the molten mixture manipulated to attempt to make it as uniform as possible. After 10 minutes of heating, the flask was allowed to cool to room temperature and was charged with ice (~60 mL) and diethyl ether (~80 mL) and stirred vigorously. The biphasic mixture was transferred to a separatory funnel, the organic layer washed with brine, then dried over $MgSO_4$, filtered and concentrated to a total volume of ~25 mL. The ethereal layer was added to a 100 mL round bottom flask, a stir bar was added and the flask was cooled to 0 °C in an ice bath. Ammonia (NH_4OH, 28% aq, 5mL) was added with vigorous stirring and an emulsion formed. After the emulsion had subsided, brine (~20 mL) and diethyl ether (~20 mL) were added and the mixture transferred to a separatory funnel. The organic phase was separated, dried over $MgSO_4$ and concentrated to give the title compound as a stiff syrup that solidified on standing (0.782g). ^{1}H NMR (400MHz, DMSO-d_6) δ (ppm) = 2.24 (p, 2H, J = 6.5 Hz), 3.12 (t, 2H, J = 6.5 Hz), 3.66 (t, 2H, J = 6.5 Hz), 6.91 (s, 2H).

Example 3.15: 3-(Tritylthio)propane-1-sulfonamide.

To a stirred solution of triphenylmethanethiol (0.276g) in *N*,*N*-dimethyl formamide at 0 °C was added sodium hydride (0.04g, 1 equiv). After effervescence had ceased, 3-bromopropane-1-sulfonamide (0.100g, 0.5 equiv) was added as a solid in a single portion and the reaction was allowed to warm to room temperature. Progress of the reaction was monitored by HPLC-MS and TLC (40% EtOAc in hexanes). After 2 h, the reaction was quenched with water (~0.5 mL) and concentrated on a rotovap at high-vacuum. The resulting oil was partitioned between ethyl acetate and brine, transferred to a separatory funnel and the organic phase was washed with brine, dried over $MgSO_4$, concentrated and purified by flash chromatography (5-50% EtOAc in hexanes) to give the title compound (0.135 g)

as a white crystalline solid. ^{1}H NMR (400MHz, CD_3OD) δ (ppm) = 1.77-1.85 (m, 2H), 2.35 (t, 2H, *J* = 6.5 Hz), 2.95-2.99 (t, 2H, *J* = 6.5 Hz), 7.22-7.33 (m, 9H), 7.40-7.45 (m, 6H).

Example 3.16: (6*S*,9*S*,12*S*,*E*)-9-*tert*-Butyl-12-isopropyl-2,2,5,11,14-pentamethyl-4,7,10-trioxo-6-(2-phenylpropan-2-yl)-3-oxa-5,8,11-triazapentadec-13-en-15-oic acid.

Synthesized as per Nieman J. A. *et al.* J. Nat. Prod. 2003, 66, 183-199.

Example 3.17 (*S*,*E*)-*N*-(3-Mercaptopropylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound A).

Example 3.17 was synthesized from Examples 3.15 and 3.16 according to General Procedures 9 and 12 with the inclusion of tri-isoproypsilane (2 equiv) to Procedure 14. ^{1}H NMR (400MHz, CD_3OD) δ (ppm) = 0.88 (3H, d, *J* = 6.2 Hz), 0.94 (3H, d, *J* = 6.2 Hz), 1.08 (s, 9H), 1.40 (s, 3H), 1.48 (s, 3H), 1.94 (d, 3H, *J* = 1.29 Hz), 2.03-2.16 (m, 3H), 2.41 (s, 3H), 2.67 (t, 2H, *J* = 9.76 Hz), 3.16 (s, 3H), 3.46-3.50 (m, 2H), 4.08 (br s, 1H), 4.94 (s, 1H), 5.07 (t, 1H, *J* = 10.0 Hz), 6.59 (d, 1H, *J* = 9.5 Hz), 7.32-7.37 (m, 1H), 7.41-7.48 (m, 2H), 7.50-7.57 (m, 2H).

Methods described above were used to generate the following analogous compounds.

Example 3.18: 2,2'-Disulfanediyldiethanesulfonamide.

Synthesized as described by Lemaire, H. and Rieger, M in J. Org. Chem., 1961, 1330-1331.

Example 3.19 (*S*,*E*)-*N*-(2-Mercaptoethylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound B).

To a solution of (6*S*,9*S*,12*S*,*E*)-9-*tert*-butyl-12-isopropyl-2,2,5,11,14-pentamethyl-4,7,10-trioxo-6-(2-phenylpropan-2-yl)-3-oxa-5,8,11-triazapentadec-13-en-15-oic acid (0.138 g, 2.4 equiv) in dichloromethane (4 mL) was added 2,2'-disulfanediyldiethanesulfonamide (0.028 g), di-isopropylcarbodiimide (0.044 mL, 2.4 equiv) and *N*,*N*-dimethylpyridine (0.034 g, 2.8 equiv). Stirring was continued for 16 h at which point TLC analysis (5% MeOH (with 5% AcOH) in 70/30 CH_2Cl_2/Hexanes) indicated complete consumption of the disulfanedisulfonamide. The reaction was diluted with hexanes (~5 mL), filtered to remove solids, concentrated and the resultant oil purified by flash chromatography. The chromatographically purified materials was then dissolved in dichloromethane (3 mL), a stir bar was added, then trifluoroacetic acid (0.60 mL) and tri-isopropylsilane (0.20 mL). The mixture immediately went yellow, with the color fading over 5 minutes and conversion of the material to the desired product was monitored by HPLC-MS. Upon complete conversion, the reaction was concentrated to dryness and the residue purified by flash chromatography (0-15% MeOH (containing 5% AcOH) in 80/20 CH_2Cl_2/hexanes). HPLC-MS showed this isolate to be a mixture of free thiol and disulfide. ^{1}H NMR (400MHz, CD_3OD) δ (ppm) = 0.88 (3H, d, *J* = 6.2 Hz), 0.93 (3H, d, *J* = 6.2 Hz), 1.07 (s, 9H), 1.40 (s, 3H), 1.47 (s, 3H), 1.91-2.05

(m, 5H), 2.32 (s, 3H), 2.67 (t, 2H, J = 9.76 Hz), 3.07-3.18 (m, 5H), 3.52-3.59 (m, 2H), 3.85 (s, 1H), HH 4.08 (br s, 1H), 4.93 (s, 1H), 5.09 (t, 1H, J = 10.0 Hz), 6.76 (d, 1H, J = 9.5 Hz), 7.29-7.35 (m, 1H), 7.39-7.46 (m, 2H), 7.49-7.55 (m, 2H). $C_{29}H_{48}N_4O_5S_2$ calcd. $[M+H]^+$ = 598.15 amu; found m/z = 598.16.

Example 3.20: 4-(Tritylthiomethyl)benzenesulfonamide.

To a stirred solution of triphenylmethanethiol (0.276 g, 2 equiv) in *N*,*N*-dimethylformamide (3 mL) at 0 °C was added sodium hydride (60% w/w dispersion in mineral oil, 0.04g, 2 equiv). When the effervescence had ceased, 4-(bromomethyl)benzenesulfonamide (0.125g, 1 equiv) was added in a single portion and the reaction was allowed to warm to room temperature. HPLC-MS at 20 minutes indicated that conversion was complete. The reaction was quenched with acetic acid (~0.2 mL), concentrated to dryness *in vacuo* and the subsequent residue partitioned between ethyl acetate and brine. The organic layer was separated, dried over $MgSO_4$, filtered, concentrated and purified by flash chromatography (0-50% ethyl acetate in hexanes). Fractions containing the desired material were concentrated to dryness to furnish the desired compound as a colorless solid (0.200g). ^{1}H NMR (400MHz, DMSO-d_6) δ (ppm) = 3.38 (s, 2H), 7.24-7.35 (m, 7H), 7.36-7.44 (m, 12H), 7.67-7.73 (m, 2H).

Example 3.21 (*S*,*E*)-*N*-(4-(Mercaptomethyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound C).

Title compound prepared from Examples 3.16 and 3.20 according to General Procedures 9 and 12 ^{1}H NMR (400MHz, CD_3OD) δ (ppm) = 0.88 (d, 3H, J = 6.2 Hz), 0.91 (d, 3H, J = 6.2 Hz), 1.06 (s, 9H), 1.38 (s, 3H), 1.47 (s, 3H), 1.86 (s, 3H), 1.99-2.05 (m, 1H), 2.41 (s, 3H), 2.67 (t, 2H, J = 9.76 Hz), 3.14 (s, 3H), 3.80 (s, 2H), HH 4.10 (br s, 1H), 4.93 (s, 1H), 5.00 (t, 1H, J = 10.0 Hz), 6.54 (d, 1H, J = 9.5 Hz), 7.30-7.51 (m, 5H), 7.52-7.58 (m, 2H), 7.90-7.97 (m, 2H). $C_{34}H_{50}N_4O_5S_2$ calcd. $[M+H]^+$ = 659.25 amu; found m/z = 659.37.

Example 3.22 (*S*,*E*)-2,5-Dimethyl-*N*-tosyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound D).

Title compound was prepared from Example 3.16 and tosylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400MHz, CD_3OD) δ (ppm) = 0.88-0.94 (m, 6H), 1.06 (s, 9H), 1.35 (s, 3H), 1.45 (s, 3H), 1.86 (s, 3H), 2.02-2.11 (m, 1H), 2.44 (s, 3H), 2.51 (s, 3H), 3.17 (s, 3H), HH 4.35 (s,

1H), 4.89-4.99 (m, 2H), 6.48 (d, 1H, J = 9.5 Hz), 7.30-7.43 (m, 4H), 7.43-7.50 (m, 2H), 7.51-7.57 (m, 2H). $C_{34}H_{50}N_4O_5S$ calcd. $[M+H]^+$ = 627.15 amu; found m/z = 627.31.

Example 3.23 (*S*,*E*)-2,5-dimethyl-*N*-(methylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound E).

Title compound was prepared from Example 3.16 and methanesulfonamide using General Procedures 9 and 12. ^{1}H NMR (400MHz, CD_3OD) δ (ppm) = 0.87-0.98 (m, 6H), 1.09 (s, 9H), 1.40 (s, 3H), 1.49 (s, 3H), 1.97 (s, 3H), 2.03-2.13 (m, 1H), 2.52 (s, 3H), 2.67 (t, 2H, J = 9.76 Hz), 3.18 (s, 3H), 3.31 (s, 3H), 4.38 (s, 1H), 4.94 (d, 1H, J = 8.2 Hz), 5.07 (t, 1H, J = 10.0 Hz), 6.54 (d, 1H, J = 9.5 Hz), 7.30-7.40 (m, 1H), 7.40-7.51 (m, 2H), 7.51-7.59 (m, 2H). $C_{28}H_{46}N_4O_5S$ calcd. $[M+H]^+$ = 551.30 amu; found m/z = 551.34.

Example 3.24 (*S*,*E*)-2,5-Dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enoic acid (Compound F).

The title compound was synthesized using methods as described by Nieman *et al.* in J. Nat. Prod. 2003, 66, 183-199.

Example 3.25: (*S*,*E*)-*N*-(Mesitylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and mesitylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.60-7.55 (m, 2H), 7.47 (m, 2H), 7.37 (m, 1H), 7.03 (s, 2H), 6.50 (d, J = 6 Hz, 1H), 5.06-4.91 (m, 3H), 4.34 (s, 1H), 3.17 (s, 3H), 2.68 (s, 6H), 2.51 (s, 3H), 2.31 (s, 3H), 2.07 (m, 6.6 Hz, 2H), 1.87 (s, 3H), 1.48 (s, 3H), 1.36 (s, 3H), 1.09-1.04 (m, J = 16.8 Hz, 10H), 0.92 (t, J = 6.3 Hz, 6H). $C_{36}H_{54}N_4O_5S$ calcd. m/z = 654.38 found $[M+H]^+$ = 655.03.

Example 3.26: (*S*,*E*)-2,5-Dimethyl-*N*-(4-(trifluoromethoxy)phenylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 4-trifluoromethoxyphenylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.16 (dd, J = 8.7, 1.4 Hz, 1H), 7.69-7.28 (m, 4H), 6.52 (d, J = 9.2 Hz, 1H), 5.02-4.95 (m, 1H), 4.92 (s, 0H), 4.35 (s, 1H), 3.17 (s, 1H), 2.51 (s, 1H), 2.05 (ddd, J = 15.9, 10.9, 3.7 Hz, 1H), 1.87 (s, 1H), 1.47 (s, 1H), 1.36 (s, 1H), 1.07 (s, 4H), 0.91 (t, J = 6.1 Hz, 3H). $C_{34}H_{47}F_3N_4O_6S$ calcd. m/z = 696.32 found $[M+H]^+$ = 697.26.

Example 3.27: (*S*,*E*)-*N*-(Benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Figure 7, Compound 14).

Title compound was prepared from Example 3.16 and benzylsulfonamide using General Procedures 9 and 12 ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.56 (d, J = 7.9 Hz, 2H), 7.47 (t, J = 7.3 Hz,

2H), 7.38 (brs, 6H), 6.39 (d, J = 9.4 Hz, 1H), 5.06 (t, J = 10.0 Hz, 1H), 4.93 (s, 1H), 4.75 (s, 2H), 4.36 (s, 1H), 3.13 (s, 3H), 2.51 (s, 3H), 2.06-1.95 (m, 4H), 1.48 (s, 3H), 1.39 (s, 3H), 1.09 (s, 9H), 0.90 (t, J = 6.2 Hz, 6H). $C_{34}H_{47}F_3N_4O_6S$ calcd. m/z = 626.35 found $[M+H]^+$ = 626.99.

Example 3.28: (*S*,*E*)-2,5-Dimethyl-*N*-(2,4,6-triisopropylphenylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2,4,6-tri-isopropylphenylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.61-7.53 (m, 2H), 7.47 (t, J = 7.8 Hz, 2H), 7.41-7.33 (m, 1H), 7.27 (s, 2H), 6.50 (dd, J = 9.6, 1.8 Hz, 1H), 5.05 (t, J = 10.0 Hz, 1H), 4.92 (s, 1H), 4.43-4.26 (m, 3H), 3.16 (s, 3H), 2.94 (dd, J = 14.3, 7.4 Hz, 1H), 2.51 (s, 3H), 2.07-1.99 (m, 2H), 1.90 (d, J = 1.4 Hz, 3H), 1.48 (s, 4H), 1.39 (s, 3H), 1.33-1.22 (m, 18H), 1.11 (s, 2H), 1.06 (s, 9H), 0.91 (t, J = 6.0 Hz, 7H). $C_{42}H_{66}N_4O_5S$ calcd. m/z = 738.48 found $[M+H]^+$ = 738.10.

Example 3.29: (*S*,*E*)-*N*-(4-*tert*-Butylphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 4-*tert*-butylphenylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.98 (d, J = 8.6 Hz, 2H), 7.64 (d, J = 8.6 Hz, 2H), 7.55 (d, J = 7.9 Hz, 2H), 7.47 (t, J = 7.7 Hz, 3H), 7.37 (t, J = 7.1 Hz, 1H), 6.48 (dd, J = 9.6, 1.8 Hz, 1H), 4.99 (t, J = 10.0 Hz, 1H), 4.92 (s, 1H), 4.35 (s, 1H), 3.16 (s, 3H), 2.51 (s, 3H), 1.87 (d, J = 1.4 Hz, 3H), 1.47 (s, 3H), 1.38 (s, 10H), 1.06 (s, 9H), 0.91 (t, J = 6.2 Hz, 7H). $C_{42}H_{66}N_4O_5S$ calcd. m/z = 668.40 found $[M+H]^+$ = 669.28.

Example 3.30: (*S*,*E*)-*N*-(4-Chlorophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 4-chlorophenylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.03 (d, J = 8.7 Hz, 2H), 7.60 (d, J = 8.7 Hz, 2H), 7.57-7.51 (m, 2H), 7.47 (dd, J = 8.6, 6.9 Hz, 2H), 7.42-7.32 (m, 1H), 6.50 (dd, J = 9.2, 1.7 Hz, 1H), 4.96 (dd, J = 10.9, 9.1 Hz, 2H), 4.92 (s, 1H), 4.35 (s, 1H), 3.17 (s, 3H), 2.51 (s, 3H), 2.14-2.03 (m, 1H), 2.01 (s, 1H), 1.87 (d, J = 1.4 Hz, 3H), 1.46 (s, 3H), 1.36 (s, 3H), 1.07 (s, 9H), 0.91 (dd, J = 6.5, 4.6 Hz, 7H). $C_{33}H_{47}ClN_4O_5S$ calcd. m/z = 646.30 found $[M+H]^+$ = 647.20.

Example 3.31: (*S*,*E*)-*N*-(3-Cyanophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 3-cyanophenylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.38 (s, 1H), 8.31 (dt, J = 8.0, 1.5 Hz, 1H), 8.02-7.92 (m, 1H), 7.75 (t, J = 7.9 Hz, 1H), 7.53 (d, J = 1.2 Hz, 1H), 7.48 (dd, J = 8.6, 6.9

Hz, 2H), 7.43-7.33 (m, 1H), 6.55 (dd, J = 9.3, 1.7 Hz, 1H), 4.93 (d, J = 5.4 Hz, 2H), 4.35 (s, 1H), 3.18 (s, 3H), 2.51 (s, 3H), 2.15-1.98 (m, 2H), 1.87 (d, J = 1.4 Hz, 3H), 1.45 (s, 3H), 1.32 (s, 3H), 1.07 (s, 9H), 0.92 (dd, J = 6.6, 3.9 Hz, 7H). $C_{34}H_{47}N_5O_5S$ calcd. m/z = 637.33 found $[M+H]^+$ = 638.00

Example 3.32: (*S*,*E*)-2,5-Dimethyl-*N*-(2-nitrophenylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2-nitrophenylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.36-8.27 (m, 1H), 7.82 (dd, J = 5.9, 3.8 Hz, 3H), 7.61-7.51 (m, 2H), 7.47 (dd, J = 8.6, 6.9 Hz, 2H), 7.42-7.31 (m, 1H), 6.63 (dd, J = 9.5, 1.7 Hz, 1H), 5.03 (t, J = 10.0 Hz, 1H), 4.93 (s, 1H), 4.36 (s, 1H), 3.18 (s, 3H), 2.51 (s, 3H), 2.12-2.01 (m, 1H), 1.88 (d, J = 1.4 Hz, 3H), 1.48 (s, 3H), 1.37 (s, 3H), 1.06 (s, 9H), 0.97-0.86 (m, 6H). C34H47N5O5S calcd. m/z = 657.32 found $[M+H]^+$ = 658.21.

Example 3.33: (*S*,*E*)-*N*-(4-Methoxy-2-nitrophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound Was prepared from Example 3.16 and 2-nitro-4-methoxyphenylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.24 (d, J = 8.9 Hz, 1H), 7.59-7.51 (m, 2H), 7.47 (t, J = 7.6 Hz, 2H), 7.44-7.25 (m, 4H), 6.60 (dd, J = 9.2, 1.7 Hz, 1H), 5.03 (t, J = 10.0 Hz, 1H), 4.93 (s, 1H), 4.36 (s, 1H), 3.97 (s, 3H), 3.18 (s, 3H), 2.51 (s, 3H), 2.13-2.02 (m, 1H), 1.89 (d, J = 1.4 Hz, 3H), 1.48 (s, 3H), 1.38 (s, 3H), 1.11 (s, 2H), 1.06 (s, 9H), 0.99-0.88 (m, 6H). $C_{34}H_{49}N_5O_8S$ calcd. m/z = 687.33 found $[M+H]^+$ = 689.23.

Example 3.34: 4-(*N*-((*S*,*E*)-2,5-Dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enoyl)sulfamoyl)-3-nitrobenzamide.

Title compound was prepared from Example 3.16 and 3-nitro-4-sulfamoylbenzamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.35 (d, J = 8.0 Hz, 1H), 8.22 (d, J = 8.0 Hz, 2H), 7.59-7.51 (m, 2H), 7.47 (t, J = 7.6 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 6.70-6.57 (m, 1H), 5.04 (t, J = 10.0 Hz, 1H), 4.94 (s, 1H), 4.37 (s, 1H), 3.17 (s, 3H), 2.52 (s, 3H), 2.05 (ddd, J = 10.3, 7.4, 5.5 Hz, 1H), 1.87 (d, J = 1.4 Hz, 3H), 1.48 (s, 3H), 1.38 (s, 3H), 1.06 (s, 9H), 0.92 (dd, J = 14.7, 6.8 Hz, 6H). $C_{34}H_{48}N_6O_8S$ calcd. m/z = 700.33 found $[M+H]^+$ = 701.28.

Example 3.35: (*S*,*E*)-*N*-(4-Methoxyphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 4-methoxyphenylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.97 (d, J = 9.0 Hz, 2H), 7.54 (d, J = 7.5 Hz, 2H), 7.46 (t, J = 7.6 Hz, 2H), 7.36 (t, J = 7.2 Hz, 1H), 7.06 (d, J = 9.0 Hz, 2H), 6.48 (dd, J =

9.3, 1.9 Hz, 1H), 4.97 (t, J = 9.9 Hz, 1H), 4.92 (s, 1H), 4.22 (s, 1H), 3.89 (s, 3H), 3.15 (s, 3H), 2.46 (s, 3H), 2.10-1.99 (m, 2H), 1.86 (d, J = 1.4 Hz, 3H), 1.46 (s, 3H), 1.36 (s, 3H), 1.06 (s, 9H), 0.94-0.84 (m, 6H). $C_{34}H_{50}N_4O_6S$ calcd. m/z = 642.35 found $[M+H]^+$ = 643.31.

Example 3.36: (*S*,*E*)-2,5-Dimethyl-*N*-(4-(2,2,2-trifluoroacetamido)phenylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Figure 7, Compound 23).

Title compound was prepared from Example 3.16 and 2,2,2-trifluoro-*N*-(4-sulfamoylphenyl)acetamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.06 (d, J = 8.9 Hz, 2H), 7.88 (d, J = 8.9 Hz, 2H), 7.52 (d, J = 7.1 Hz, 2H), 7.49-7.40 (m, 3H), 7.35 (dd, J = 8.1, 6.1 Hz, 1H), 6.47 (dd, J = 9.2, 1.8 Hz, 1H), 4.33 (s, 1H), 3.15 (s, 3H), 2.48 (s, 3H), 2.13-1.96 (m, 2H), 1.85 (d, J = 1.4 Hz, 3H), 1.43 (s, 3H), 1.33 (s, 3H), 1.04 (s, 9H), 0.89 (dd, J = 6.8, 4.7 Hz, 6H). $C_{35}H_{48}F_3N_5O_6S$ calcd. m/z = 723.33 found $[M+H]^+$ = 724.08.

Example 3.37: (*S*,*E*)-*N*-(4-Aminophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound 886).

Title compound was prepared from Example 3.16 and 2,2,2-trifluoro-*N*-(4-sulfamoylphenyl)acetamide using General Procedures 2, 3 and 7 ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.71 (d, J = 8.8 Hz, 2H), 7.55 (d, J = 7.6 Hz, 2H), 7.47 (d, J = 6.9 Hz, 2H), 7.37 (t, J = 6.8 Hz, 1H), 6.67 (d, J = 8.8 Hz, 2H), 6.44 (dd, J = 9.2, 1.6 Hz, 1H), 4.97 (t, J = 9.7 Hz, 1H), 4.92 (s, 1H), 4.36 (s, 1H), 3.16 (s, 3H), 2.51 (s, 3H), 2.16-2.00 (m, 1H), 1.87 (d, J = 1.4 Hz, 3H), 1.46 (s, 3H), 1.37 (s, 3H), 1.07 (s, 9H), 0.92 (d, J = 6.4 Hz, 3H), 0.91 (d, J = 6.3 Hz, 3H). $C_{33}H_{49}N_5O_5S$ calcd. m/z = 627.35 found $[M+H]^+$ = 628.35.

Example 3.38: (*S*,*E*)-2,5-Dimethyl-*N*-(phenylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and phenylsulfonamide using General Procedures 2, and 7. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.06-7.95 (m, 2H), 7.63-7.40 (m, 8H), 7.40-7.30 (m, 1H), 6.53 (dd, J = 9.3, 1.6 Hz, 1H), 5.05-4.95 (m, 1H), 4.22 (s, 1H), 3.14 (s, 3H), 2.45 (s, 3H), 2.09-1.95 (m, 1H), 1.85 (d, J = 1.4 Hz, 3H), 1.46 (s, 3H), 1.36 (s, 3H), 1.06 (s, 9H), 0.89 (dd, J = 11.9, 6.5 Hz, 7H). $C_{33}H_{48}N_4O_5S$ calcd. m/z = 612.33 found $[M+H]^+$ = 613.06 .

Example 3.39: (*S*,*E*)-*N*-(*N*-(2-Fluorobenzyl)sulfamoyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

2-Fluorobenzylsulfamamide was prepared from 2-fluorobenzylamine according to General Procedure 14; the title compound was prepared from Example 3.16 and 2-fluorobenzylsulfamamide

using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.63-7.41 (m, 6H), 7.41-7.26 (m, 3H), 7.14 (td, J = 7.5, 1.2 Hz, 1H), 7.07 (ddd, J = 9.5, 8.2, 1.1 Hz, 1H), 6.37 (dd, J = 9.4, 1.7 Hz, 1H), 5.07-4.97 (m, 1H), 4.37 (s, 1H), 4.33 (s, 2H), 3.15 (s, 3H), 2.51 (s, 3H), 2.10-1.97 (m, 1H), 1.83 (d, J = 1.4 Hz, 3H), 1.49 (s, 3H), 1.39 (s, 3H), 1.09 (s, 9H), 0.97-0.84 (m, 6H). $C_{34}H_{50}FN_5O_5S$ calcd. m/z = 659.35 found $[M+H]^+$ = 660.28.

Example 3.40: (*S*,*E*)-2,5-Dimethyl-*N*-(piperidin-1-ylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Piperidine-1-sulfonamide was synthesized from piperidine according to General Procedure 14; the title compound was prepared from Example 3.16 and piperidine-1-sulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.55 (d, J = 1.2 Hz, 1H), 7.47 (t, J = 7.6 Hz, 3H), 7.42-7.29 (m, 1H), 6.48 (dd, J = 9.7, 1.8 Hz, 1H), 5.05 (t, J = 10.0 Hz, 1H), 4.39 (s, 1H), 3.18 (s, 3H), 2.52 (s, 3H), 2.07 (d, J = 10.5 Hz, 1H), 1.96 (d, J = 1.4 Hz, 3H), 1.61 (ddd, J = 20.0, 10.3, 5.4 Hz, 9H), 1.49 (s, 4H), 1.39 (s, 3H), 1.09 (s, 9H), 0.99-0.84 (m, 9H). $C_{32}H_{53}N_5O_5S$ calcd. m/z = 619.38 found $[M+H]^+$ = 620.38.

Example 3.41: (*S*,*E*)-2,5-Dimethyl-*N*-(o-tolylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2-toluenesulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.10 (dd, J = 8.0, 1.4 Hz, 1H), 7.60-7.33 (m, 11H), 6.52 (dd, J = 9.6, 1.7 Hz, 1H), 5.04-4.90 (m, 2H), 4.35 (s, 1H), 3.18 (s, 3H), 2.67 (s, 3H), 2.51 (s, 3H), 2.15-2.03 (m, 2H), 2.01 (s, 1H), 1.87 (d, J = 1.4 Hz, 3H), 1.46 (s, 3H), 1.35 (s, 3H), 1.07 (s, 9H), 0.92 (t, J = 6.3 Hz, 6H). $C_{34}H_{50}N_4O_5S$ calcd. m/z = 626.35 found $[M+H]^+$ = 627.05.

Example 3.42: (*S*,*E*)-*N*-(4-Bromophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 4-bromophenylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.95 (d, J = 8.3 Hz, 2H), 7.76 (d, J = 8.0 Hz, 2H), 7.55 (d, J = 7.5 Hz, 2H), 7.47 (dd, J = 8.6, 6.9 Hz, 2H), 7.41-7.29 (m, 1H), 6.51 (d, J = 9.0 Hz, 1H), 4.35 (s, 1H), 3.16 (s, 3H), 2.50 (s, 3H), 2.06 (dt, J = 10.7, 6.3 Hz, 1H), 1.87 (s, 3H), 1.46 (s, 3H), 1.36 (s, 3H), 1.07 (s, 9H), 0.91 (dd, J = 6.9, 4.9 Hz, 8H). $C_{33}H_{47}BrN_4O_5S$ calcd. m/z = 690.25 found $[M+H]^+$ = 691.17, 693.18.

Example 3.43: (*S*,*E*)-2,5-Dimethyl-*N*-(naphthalen-2-ylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2-naphthylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.69-8.62 (m, 1H), 8.47 (d, J = 8.2 Hz, 1H), 8.14-7.95 (m, 5H), 7.71 (dddd, J = 18.4, 8.2, 6.9, 1.4 Hz, 2H), 7.57-7.50 (m, 2H), 7.46 (dd, J = 8.6, 6.9 Hz, 2H), 7.42-7.33 (m, 1H), 6.50 (dd, J = 9.3, 1.5 Hz, 1H), 4.92-4.87 (m, 1H), 4.34 (s, 1H), 3.16 (s, 3H), 2.50 (s, 3H), 2.13-1.99 (m, 1H), 1.85 (d, J = 1.4 Hz, 3H), 1.44 (s, 3H), 1.34 (s, 3H), 1.04 (s, 9H), 0.90 (dd, J = 6.6, 4.0 Hz, 6H). $C_{37}H_{50}N_4O_5S$ calcd. m/z = 662.35 found $[M+H]^+$ = 663.32.

Example 3.44: Methyl 4-(*N*-((*S*,*E*)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enoyl)sulfamoyl)benzoate.

Title compound was prepared from Example 3.16 and 4-carboxymethylphenylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.24-8.10 (m, 4H), 7.58-7.50 (m, 2H), 7.47 (dd, J = 8.6, 6.9 Hz, 2H), 7.41-7.33 (m, 1H), 6.52 (dd, J = 9.2, 1.6 Hz, 1H), 4.35 (s, 1H), 3.97 (s, 3H), 3.18 (s, 3H), 2.50 (s, 3H), 2.15-2.00 (m, 1H), 1.86 (d, J = 1.4 Hz, 3H), 1.45 (s, 3H), 1.35 (s, 3H), 1.07 (s, 9H), 0.91 (dd, J = 6.7, 3.8 Hz, 6H). $C_{35}H_{50}N_4O_7S$ calcd. m/z = 670.34 found $[M+H]^+$ = 671.10.

Example 3.45: (*S*,*E*)-2,5-Dimethyl-*N*-(*N*-(2-(trifluoromethyl)benzyl)sulfamoyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2-trifluoromethylbenzylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.78 (d, J = 7.9 Hz, 1H), 7.74-7.67 (m, 1H), 7.64 (dd, J = 8.1, 6.7 Hz, 1H), 7.60-7.52 (m, 2H), 7.48 (dd, J = 8.5, 6.8 Hz, 4H), 7.42-7.33 (m, 1H), 6.48-6.40 (m, 1H), 5.11-5.02 (m, 1H), 4.45 (s, 2H), 4.37 (s, 1H), 3.17 (s, 3H), 2.52 (s, 3H), 2.11-1.99 (m, 2H), 1.92 (d, J = 1.4 Hz, 3H), 1.49 (s, 3H), 1.40 (s, 3H), 1.09 (s, 9H), 0.92 (dd, J = 9.3, 6.7 Hz, 6H). $C_{35}H_{50}F_3N_5O_5S$ calcd. m/z = 709.35 found $[M+H]^+$ = 710.02.

Example 3.46: (4*S*,*E*)-*N*-(Hexan-2-ylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and hexane-2-sulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.56-7.48 (m, 2H), 7.42 (t, J = 7.8 Hz, 2H), 7.31 (t, J = 7.3 Hz, 1H), 6.58-6.50 (m, 1H), 5.05 (t, J = 10.0 Hz, 1H), 4.92 (s, 1H), 3.84 (s, 1H), 3.65 (dt, J = 10.8, 4.3 Hz, 1H), 3.14 (s, 3H), 2.32 (s, 3H), 2.09-1.96 (m, 2H), 1.93 (d, J = 1.4 Hz, 3H), 1.61-1.27 (m, 3H), 1.06 (s, 9H), 0.98-0.90 (m, 6H), 0.87 (d, J = 6.5 Hz, 3H). $C_{33}H_{56}N_4O_5S$ calcd. m/z = 620.40 found $[M+H]^+$ = 621.55.

Example 3.47: (*S*,*E*)-*N*-(2-Methoxyethylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2-methoxyethanesulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.56 (d, J = 7.8 Hz, 2H), 7.47 (t, J = 7.6 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 6.51 (d, J = 9.4 Hz, 1H), 5.07 (t, J = 10.0 Hz, 1H), 4.95 (s, 1H), 4.33 (s, 1H), 3.82 (t, J = 5.8 Hz, 2H), 3.70 (q, J = 5.2 Hz, 2H), 3.18 (s, 3H), 2.50 (s, 3H), 2.18-2.00 (m, 1H), 1.95 (d, J = 1.4 Hz, 3H), 1.49 (s, 3H), 1.39 (s, 3H), 1.09 (s, 9H), 0.93 (dd, J = 14.8, 6.6 Hz, 6H). $C_{30}H_{50}N_4O_6S$ calcd. m/z = 594.35 found $[M+H]^+$ = 595.44.

Example 3.48: (*S*,*E*)-*N*-(Cyclopentylmethylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and cyclopentylmethanesulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.61-7.52 (m, 2H), 7.48 (dd, J = 8.6, 6.9 Hz, 2H), 7.38 (t, J = 7.4 Hz, 1H), 6.54 (dd, J = 9.4, 1.7 Hz, 1H), 5.06 (t, J = 10.0 Hz, 1H), 4.94 (s, 1H), 4.37 (s, 1H), 3.52 (dd, J = 7.0, 5.4 Hz, 3H), 3.18 (s, 3H), 2.52 (s, 3H), 2.35 (p, J = 8.1 Hz, 1H), 2.16-1.89 (m, 6H), 1.77-1.53 (m, 4H), 1.49 (s, 3H), 1.45-1.26 (m, 5H), 1.09 (s, 9H), 0.93 (dd, J = 11.3, 6.7 Hz, 6H). $C_{33}H_{54}N_4O_5S$ calcd. m/z = 618.38 found $[M+H]^+$ = 619.54.

Example 3.49: (*S*)-Methyl 2-(*tert*-butoxycarbonyl(methyl)amino)-3-(4-cyanophenyl)-3-methylbutanoate.

To a mixture of the methyl ester of Example 3.51 (0.06g, 0.15mmol), tris(dibenzylideneacetone)dipalladium(0) (0.014g, 0.015mmol), 1,1'-bis(diphenylphosphino)ferrocene (0.02g, 0.25 equiv), magnesium acetate (0.013g, 0.06mmol), zinc dust (0.004g, 0.06mmol) and zinc cyanide (0.0264g, 0.225mmol) under a bath of nitrogen was added *N*,*N*-dimethylformamide/water (0.8/0.08mL). The reaction was sparged with nitrogen gas, then the vial was sealed and immersed in an oil bath at 105 °C. The reaction was allowed to stir overnight and allowed to cool to room temperature. HPLC-MS analysis indicated good conversion to the desired product. The reaction was concentrated at reduced pressure, suspended in CH_2Cl_2 and the resulting suspension purified by silica gel chromatography (15-25% EtOAc in Hexanes) to yield the final compound as a colorless oil (0.036g, 69%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.69-7.35 (m, 4H), 5.24 (s, 1H), 3.54 (s, 3H), 2.74 (s, 3H), 1.51 (s, 3H), 1.45-1.25 (m, 12H).

Example 3.50: (*S*)-Methyl 2-(*tert*-butoxycarbonyl(methyl)amino)-3-(4-((*tert*-butoxycarbonylamino)methyl)phenyl)-3-methylbutanoate.

To a solution of the benzonitrile (0.300g, 0.87mmol) in methanol/acetic acid (10:1, 9 mL) in a shaker vessel was added palladium black. The flask was charged with hydrogen gas at 60psi and the shaker turned on for 24h. At that time, the vessel was purged of H2 under reduced pressure. The reaction was diluted with methanol and the suspension filtered through a Celite® pad. The filtrate was

concentrated to a slightly yellow oil and re-dissolved in dichloromethane (5mL). *t*-Butyl dicarbonate (0.524g, 2.0 equiv) and triethylamine (0.846mL, 5 equiv) were added to the solution at 0 °C with stirring. The reaction was allowed to stir for 3h at which time HPLC-MS indicated complete consumption of the amine. The reaction was concentrated under reduced pressure and purified by silica gel chromatography (diethyl ether in hexanes, 15-30%) to yield the title compound as a colorless oil (0.232g, 60%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.38 (dd, *J* = 16.6, 8.0 Hz, 2H), 7.23 (d, *J* = 7.7 Hz, 2H), 5.27 (s, 1H), 4.31 (s, 2H), 3.61 (s, 3H), 2.78 (s, 3H), 1.50-1.61 (m, 6H), 1.47 (d, *J* = 15.2 Hz, 18H).

Example 3.51: (*S*)-3-(4-Bromophenyl)-2-(*tert*-butoxycarbonyl(methyl)amino)-3-methylbutanoic acid.

To a stirred solution of (*S*)-methyl 3-(4-bromophenyl)-2-(*tert*-butoxycarbonyl(methyl)amino)-3-methylbutanoate (0.710g, 1.77mmol) in 1,4 dioxane (4 mL) was added water (1mL) (2mL) and lithium hydroxide monohydrate (0.367g, 8.9mmol). The reaction was heated to 50 °C and monitored by HPLC for completion. The reaction was cooled to room temperature, acidified to pH 3 with 1M citric acid and concentrated to near dryness under reduced pressure. The residue was taken up in ~20mL ethyl acetate, washed with brine, dried over $MgSO_4$, filtered and concentrated to give analytically pure material that was used without further manipulation. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.44 (d, *J* = 8.3 Hz, 2H), 7.33 (d, *J* = 8.3 Hz, 2H), 5.18 (s, 1H), 2.71 (s, 3H), 1.60-1.42 (m, 15H).

Example 3.52: (*S*)-3-(4-Azidophenyl)-2-(*tert*-butoxycarbonyl(methyl)amino)-3-methylbutanoic acid.

To an open pressure tube containing a magnetic stir bar was added Example 3.51 (0.690g, 1.8mmol), copper(I) iodide (0.034g, 0.18mmol), sodium azide (0.350g, 5.4mmol), N^1,N^2-dimethylethane-1,2-diamine (0.029mL, 0.27mmol), sodium ascorbate (0.036g, 0.18mmol), sodium hydroxide (0.072g, 1.8mmol), ethanol (6mL) and water (1mL). The suspension was sparged with nitrogen gas, the vessel was sealed and immersed in an oil bath at 105 °C with vigorous stirring. The course of reaction was monitored by HPLC-MS over the course of 24h at which time little starting material remained. The reaction was diluted with ethyl acetate (~20mL) and washed with brined. The aqueous layer was extracted 2× with ~20 mL ethyl acetate. The organic layers were combined, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (20-65% EtOAc (containing 2%v/v AcOH) in hexanes) to give the title compound as a colorless oil (0.475g, 75%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.44 (d, *J* = 8.6 Hz, 2H), 6.99 (dd, *J* = 9.0, 3.4 Hz, 2H), 5.24 (s, 1H), 2.71 (s, 3H), 1.63-1.38 (m, 18H).

Example 3.53: (*S*,*E*)-*N*-(Benzylsulfonyl)-4-((*S*)-2-((*S*)-3-(4-cyanophenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enamide.

Title compound was prepared from Example 3.49 and (*S*,*E*)-4-((*S*)-2-amino-*N*,3,3-trimethylbutanamido)-*N*-(benzylsulfonyl)-2,5-dimethylhex-2-enamide using General Procedures 10, 11 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.83 (d, J = 8.2 Hz, 2H), 7.73 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 2.6 Hz, 5H), 6.39 (dd, J = 9.2, 1.8 Hz, 1H), 5.04 (t, J = 10.1 Hz, 1H), 4.91 (s, 1H), 4.75 (s, 2H), 4.34 (s, 1H), 3.12 (s, 3H), 2.54 (s, 3H), 2.05-1.97 (m, 2H), 1.95 (d, J = 1.5 Hz, 3H), 1.52 (s, 3H), 1.41 (s, 3H), 1.09 (s, 9H), 0.91 (dd, J = 11.2, 4.8 Hz, 6H). $C_{35}H_{49}N_5O_5S$ calcd. m/z = 651.35 found $[M+H]^+$ = 652.4.

Example 3.54: (*S*,*E*)-4-((*S*)-2-((*S*)-3-(4-(Aminomethyl)phenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-*N*-(benzylsulfonyl)-2,5-dimethylhex-2-enamide.

Title compound was prepared from Example 3.50 and (*S*,*E*)-4-((*S*)-2-amino-*N*,3,3-trimethylbutanamido)-*N*-(benzylsulfonyl)-2,5-dimethylhex-2-enamide using General Procedures 10, 11 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.63 (t, J = 8.8 Hz, 2H), 7.54 (d, J = 8.3 Hz, 2H), 7.49-7.43 (m, 3H), 7.39 (m, 2H), 6.39 (d, J = 9.4 Hz, 1H), 5.05-4.97 (m, 1H), 4.75 (s, 2H), 4.35 (s, 3H), 4.16 (s, 2H), 3.14 (s, 3H), 2.54 (s, 3H), 2.03 (m, 1H), 1.95 (s, 3H), 1.51 (s, 3H), 1.39 (s, 3H), 1.31 (s, 3H), 1.09 (s, 9H), 0.98-0.81 (m, 6H).

Example 3.55: (*S*,*E*)-4-((*S*)-2-((*S*)-3-(4-Azidophenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-*N*-(benzylsulfonyl)-2,5-dimethylhex-2-enamide.

Title compound was prepared from Example 3.52 and (*S*,*E*)-4-((*S*)-2-amino-*N*,3,3-trimethylbutanamido)-*N*-(benzylsulfonyl)-2,5-dimethylhex-2-enamide using General Procedures 11 and 12. $C_{34}H_{49}N_7O_5S$ calcd. m/z = 667.35 amu; found $[M+H]^+$ = 668.4.

Example 3.56: (*S*,*E*)-4-((*S*)-2-((*S*)-3-(4-Aminophenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-*N*-(benzylsulfonyl)-2,5-dimethylhex-2-enamide.

To a stirred solution of Boc protected Example 3.55 (0.035g, 0.046mmol) in ethanol (1.6 mL) and water (0.5 mL) was added zinc dust (0.015g, 0.23 mmol) and ammonium chloride (0.025g, 0.46mmol). After 1h HPLC-MS indicated complete consumption of the starting material. The reaction was quenched with ammonium hydroxide (~0.1mL) and diluted with ethyl acetate (5mL). The reaction was filtered, the solids washed with ethyl acetate (5mL) and the biphasic filtrate transferred to a separatory funnel. The aqueous phase was washed twice with ethyl acetate (5mL) and the organic phases were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated. The reaction product was purified by silica gel chromatography (5-15% MeOH in CH_2Cl_2) to afford the Boc

protected intermediate as a colorless glass (0.027g, 66%). The intermediate was deprotected according to General Procedure 12 to give the title compound. $C_{34}H_{51}N_5O_5S$ calcd. *m/z* = 641.36 amu; found $[M+H]^+$ = 642.4.

Example 3.57: (*S*,*E*)-*N*-(Cyclohexylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and cyclohexylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.61-7.52 (m, 2H), 7.47 (dd, *J* = 8.6, 6.9 Hz, 2H), 7.36 (t, *J* = 7.5 Hz, 1H), 6.61-6.50 (m, 1H), 5.11-4.99 (m, 1H), 4.94 (s, 1H), 4.28 (s, 1H), 3.59-3.51 (m, 1H), 3.18 (s, 3H), 2.48 (s, 3H), 2.20-2.00 (m, 4H), 1.97-1.87 (m, 6H), 1.78-1.69 (m, 1H), 1.60 (td, *J* = 14.2, 10.9 Hz, 2H), 1.48 (s, 3H), 1.44-1.23 (m, 6H), 1.09 (s, 9H), 0.93 (dd, *J* = 13.7, 6.6 Hz, 7H). $C_{33}H_{54}N_4O_5S$ calcd. *m/z* = 618.38 found $[M+H]^+$ = 619.47.

Example 3.58: (*S*,*E*)-2,5-Dimethyl-*N*-(pyridin-3-ylmethylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and pyridin-3-ylmethanesulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.55 (d, *J* = 1.7 Hz, 1H), 8.48 (dd, *J* = 5.0, 1.6 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 0H), 7.55 (d, *J* = 7.6 Hz, 2H), 7.50-7.39 (m, 2H), 7.35 (s, 1H), 6.52 (dd, *J* = 9.6, 2.0 Hz, 1H), 5.05 (s, 0H), 4.94 (s, 1H), 4.64 (s, 2H), 4.19 (s, 1H), 3.11 (s, 3H), 2.45 (s, 3H), 1.91 (d, *J* = 1.5 Hz, 3H), 1.48 (s, 3H), 1.39 (s, 3H), 1.07 (s, 8H), 0.89 (dd, *J* = 15.1, 6.5 Hz, 6H). $C_{33}H_{54}N_4O_5S$ calcd. *m/z* = 627.35 found $[M+H]^+$ = 628.35.

Example 3.59: 4-(*N*-((*S*,*E*)-2,5-Dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enoyl)sulfamoyl)benzoic acid.

Title compound was prepared from Example 3.16 and methyl 4-sulfamoylbenzoate using General Procedures 9, 10 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.25-8.07 (m, 4H), 7.54 (d, *J* = 7.8 Hz, 2H), 7.47 (t, *J* = 7.6 Hz, 2H), 7.37 (t, *J* = 7.3 Hz, 1H), 6.55 (d, *J* = 9.3 Hz, 1H), 4.98 (t, *J* = 9.9 Hz, 1H), 4.92 (s, 1H), 4.36 (s, 1H), 3.16 (s, 3H), 2.51 (s, 3H), 2.06 (q, *J* = 9.0, 7.7 Hz, 1H), 1.88 (s, 3H), 1.46 (s, 3H), 1.36 (s, 3H), 1.06 (s, 9H), 0.91 (t, *J* = 6.0 Hz, 6H).

Example 3.60: (*S*,*E*)-2,5-Dimethyl-*N*-(3-(2,2,2-trifluoroacetamido)phenylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2,2,2-trifluoro-*N*-(3-sulfamoylphenyl)acetamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.49 (p, *J* = 2.2 Hz, 1H), 7.90 (dtd, *J* = 6.0, 4.8, 2.9 Hz, 2H), 7.64-7.56 (m, 1H), 7.53 (tt, *J* = 5.4, 4.3, 1.8 Hz, 2H), 7.51-7.42 (m, 2H), 7.41-7.28 (m, 1H), 6.56-6.38 (m, 1H), 4.97 (s, 1H), 4.90 (d, *J* = 3.3

Hz, 1H), 4.35 (s, 1H), 3.16 (d, *J* = 15.5 Hz, 3H), 2.49 (d, *J* = 14.2 Hz, 3H), 2.14-2.01 (m, 1H), 1.89-1.83 (m, 3H), 1.57-1.28 (m, 6H), 1.14-0.94 (m, 9H), 0.95-0.85 (m, 6H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 172.26, 168.81, 167.10, 167.00, 144.95, 141.82, 138.82, 138.47, 135.31, 130.71, 130.38, 128.91, 127.36, 126.65, 126.32, 121.39, 71.20, 66.92, 57.87, 57.78, 42.05, 35.83, 34.15, 32.66, 30.84, 29.79, 26.95, 21.39, 19.84, 19.82, 15.45, 14.03. ^{19}F NMR (377 MHz, Methanol-d_4) δ -76.96,-77.07. $C_{35}H_{48}F_3N_5O_6S$ calcd. *m/z* = 723.33 amu; found $[M+H]^+$ = 724.30, [M+Na]+ = 746.30.

Example 3.61: (*S*,*E*)-*N*-(3-Aminophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2,2,2-trifluoro-*N*-(3-sulfamoylphenyl)acetamide using General Procedures 9, 10 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.55 (d, *J* = 7.5 Hz, 2H), 7.51-7.45 (m, 2H), 7.43-7.20 (m, 4H), 6.97 (d, *J* = 8.1 Hz, 1H), 6.48 (d, *J* = 9.4 Hz, 1H), 5.02-4.89 (m, 2H), 4.36 (s, 1H), 3.17 (s, 3H), 2.50 (s, 3H), 2.14-2.00 (m, 1H), 1.88 (d, *J* = 1.4 Hz, 3H), 1.46 (s, 3H), 1.35 (s, 3H), 1.07 (s, 9H), 0.92 (d, *J* = 6.3 Hz, 3H), 0.90 (s, 3H). $C_{33}H_{49}N_5O_5S$ calcd. *m/z* = 627.35 found $[M+H]^+$ = 628.36.

Example 3.62: (*S*,*E*)-2,5-Dimethyl-*N*-(pyridin-3-ylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and pyridine-3-sulfonamide using General Procedures 2, and 7. ^{1}H NMR (400 MHz, Methanol-d_4) δ 9.18 (s, 1H), 8.80 (s, 1H), 8.46 (dt, *J* = 8.2, 1.8 Hz, 1H), 7.65 (dd, *J* = 8.1, 4.9 Hz, 1H), 7.54 (d, *J* = 7.3 Hz, 2H), 7.47 (t, *J* = 7.8 Hz, 2H), 7.37 (t, *J* = 7.3 Hz, 1H), 6.54 (d, *J* = 9.3 Hz, 1H), 5.01-4.88 (m, 2H), 4.36 (s, 1H), 3.18 (s, 3H), 2.51 (s, 3H), 2.15-2.01 (m, 1H), 1.86 (d, *J* = 1.4 Hz, 3H), 1.46 (s, 3H), 1.33 (s, 3H), 1.07 (s, 9H), 0.92 (d, *J* = 3.3 Hz, 3H), 0.91 (d, *J* = 3.5 Hz, 3H). $C_{32}H_{47}N_5O_5S$ calcd. *m/z* =613.33 found $[M+H]^+$ = 614.23.

Example 3.63: (*S*,*E*)-2,5-Dimethyl-*N*-(thiophen-2-ylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and thiophene-2-sulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.93-7.82 (m, 2H), 7.55 (d, *J* = 8.3 Hz, 1H), 7.48 (t, *J* = 7.8 Hz, 2H), 7.37 (t, *J* = 7.2 Hz, 1H), 7.15 (dd, *J* = 5.0, 3.8 Hz, 1H), 6.51 (d, *J* = 9.1 Hz, 1H), 5.02-4.93 (m, 2H), 4.36 (s, 1H), 3.18 (s, 3H), 2.51 (s, 3H), 2.15-2.01 (m, 1H), 1.89 (d, *J* = 1.4 Hz, 3H), 1.46 (s, 3H), 1.34 (s, 3H), 1.08 (s, 9H), 0.93 (d, *J* = 4.8 Hz, 3H), 0.91 (d, *J* = 4.7 Hz, 3H). $C_{31}H_{46}N_4O_5S_2$ calcd. *m/z* = 618.29 found $[M+H]^+$ = 619.24.

Example 3.64: (*S*,*E*)-*N*-(4-Hydroxyphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 4-(*tert*-butyldimethylsilyloxy)benzenesulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.89 (d, J = 8.8 Hz, 2H), 7.55 (d, J = 7.0 Hz, 1H), 7.47 (t, J = 7.6 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 6.91 (d, J = 8.9 Hz, 2H), 6.46 (d, J = 9.2 Hz, 1H), 4.97 (d, J = 10.2 Hz, 1H), 4.92 (s, 1H), 4.33 (s, 1H), 3.16 (s, 3H), 2.50 (s, 3H), 2.11-2.00 (m, 1H), 1.87 (d, J = 1.4 Hz, 3H), 1.46 (s, 3H), 1.36 (s, 3H), 1.07 (s, 9H), 0.92 (d, J = 6.5 Hz, 4H), 0.89 (d, J = 6.7 Hz, 3H). $C_{33}H_{48}N_4O_6S$ calcd. m/z = 628.33 found $[M+H]^+$ = 629.38.

Example 3.65: 4-(Tritylthiomethyl)benzonitrile

Tritylmercaptan (1.48 g, 5.36 mmol, 1.05 eq) in THF (5 mL) was added dropwise to a stirred suspension of sodium hydride (60% dispersion in mineral oil, 214 mg, 5.36 mmol, 1.05 eq) in THF (5 mL) under N2 at 0 °C. After 15 min, 4-(bromomethyl)benzonitrile (1.00g, 5.10 mmol, 1.0 eq) in THF (5 mL) was added and the reaction was allowed to come to rt. After 1 h, TLC indicated complete conversion of starting material. The reaction was quenched by adding saturated ammonium chloride, then some dH_2O. The mixture was extracted three times with ether, washed with saturated brine, dried over sodium sulfate, and concentrated to a viscous yellow oil. Purification by flash chromatography gave the title compound (1.76 g, 88%) as a light white powder. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.52 (d, J = 8.2 Hz, 2H), 7.47 (d, J = 7.1 Hz, 6H), 7.33 (t, J = 7.5 Hz, 6H), 7.26 (t, J = 7.2 Hz, 3H), 7.19 (d, J = 8.2 Hz, 2H), 3.40 (s, 2H). m/z calcd. for $C_{27}H_{21}NS$ = 391.14. Found $[M+Na]^+$ = 414.13. R_f = 0.32 (10% EtOAc/Hex).

Example 3.66: 1-(4-(Tritylthiomethyl)phenyl)cyclopropanamine.

4-(Tritylthiomethyl)benzonitrile (1.47 g, 3.75 mmol, 1.0 eq) was taken up in 40 mL THF, under N2 atmosphere, then cooled to-78 °C. To this solution was added Ti(O-*i*Pr)$_4$ (1.21 mL, 4.13 mmol, 1.1 eq), then ethylmagnesium bromide (3 M, 2.75 mL, 8.26 mmol, 2.2 eq) was added dropwise over 5 min. The dry-ice bath was removed, allowing the solution to reach rt. After 45 min at rt, BF3•Et_2O (0.93 mL, 7.51 mmol, 2.0 eq) was added to the now very dark reaction mixture. After stirring for an additional 2.5 h, the reaction was quenched with 5 mL of 2 M HCl, followed by pH adjustment to strong base with about 15 mL 2 M NaOH. Some water was added to the mixture, then it was extracted three times with 75 mL EtOAc, washed once with dH_2O, once with saturated brine, dried over sodium sulfate, and concentrated to a clear oil. The material was purified by flash chromatography to afford the title compound (680 mg, 36%) as a clear oil. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.49 (d, J = 7.8 Hz, 6H), 7.33 (t, J = 7.7 Hz, 6H), 7.26 (t, J = 7.2 Hz, 3H), 7.20 (d, J = 8.2 Hz, 2H), 7.11 (d, J = 8.2 Hz, 2H), 3.32 (s, 2H), 1.06 (dd, J = 7.9, 5.0 Hz, 2H), 0.95 (dd, J = 7.9,

4.7 Hz, 2H). *m/z* calcd. for $C_{29}H_{27}NS$ = 421.19. Found $[M+H]^+$ = 422.19. R_f = 0.21 (50% EtOAc/Hex).

Example 3.67: 2,2,2-Trifluoro-*N*-(1-(4-(tritylthiomethyl)phenyl)cyclopropyl)acetamide.

To a stirred solution of 1-(4-(tritylthiomethyl)phenyl)cyclopropanamine (680 mg, 1.61 mmol, 1.0 eq) in CH_2Cl_2 was added trifluoroacetic anhydride (0.448 mL, 3.22 mmol, 2.0 eq) and triethylamine (0.45 mL, 3.22 mmol, 2.0 eq). After two hours, TLC and HPLC indicated complete conversion of starting material. The reaction was quenched by the addition of 3 mL NaHCO3, then some dH2O was added, and the mixture was extracted three times with CH_2Cl_2. The combined organics were washed with saturated brine, dried over sodium sulfate, and concentrated to a yellow foam, giving the title compound (715 mg, 86%) in sufficient purity to move to the next step. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.48 (d, *J* = 7.7 Hz, 6H), 7.32 (t, *J* = 7.6 Hz, 6H), 7.25 (t, *J* = 7.2 Hz, 3H), 7.19 (d, *J* = 8.2 Hz, 2H), 7.10 (d, *J* = 8.3 Hz, 2H), 6.83 (s, 1H), 3.31 (s, 2H), 1.40-1.24 (m, 4H). *m/z* calcd. for $C_{31}H_{26}F_3NOS$ = 517.17. Found $[M+Na]^+$ = 540.25. R_f = 0.71 (50% EtOAc/Hex).

Example 3.68: 2,2,2-Trifluoro-*N*-(1-(4-(mercaptomethyl)phenyl)cyclopropyl)acetamide.

2,2,2-Trifluoro-*N*-(1-(4-(tritylthiomethyl)phenyl)cyclopropyl)acetamide (715 mg, 1.38 mmol, 1.0 eq) in 5 mL CH_2Cl_2 was treated with 2.5 mL TFA. After 1 min, TIPSH (0.42 mL, 2.1 mmol, 1.5 eq) was added, causing the yellow color to fade. After 30 min, TLC indicated the reaction to be complete. The mixture was concentrated, then co-evaporated once with CH_2Cl_2 and twice with toluene. The residue was purified by flash chromatography to afford the title compound (261 mg, 69%) as a white solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.35-7.23 (m, 4H), 6.87 (s, 1H), 3.74 (d, *J* = 7.6 Hz, 2H), 1.77 (t, *J* = 7.6 Hz, 1H), 1.36 (s, 4H). R_f = 0.47 (20% EtOAc/Hex).

Example 3.69: 2,2,2-Trifluoro-*N*-(1-(4-(sulfamoylmethyl)phenyl)cyclopropyl)acetamide.

To a stirred solution of 2,2,2-trifluoro-*N*-(1-(4-(mercaptomethyl)phenyl)cyclopropyl)acetamide (220 mg, 0.799 mmol, 1.0 eq) in acetonitrile were added dH2O (0.029 mL, 1.6 mmol, 2.0 eq), tetrabutylammonium chloride (110 mg, 0.40 mmol, 0.5 eq), then *N*-chlorosuccinimide (320 mg, 2.40 mmol, 3.0 eq). After 20 minutes, no starting material was visible by TLC. After 90 min, concentrated NH_4OH (0.18 mL, 3.2 mmol, 4.0 eq) was added. After 10 minutes, 1 mL of NH_4Cl was added, and the mixture was extracted three times with EtOAc. The combined organics were washed twice with dH_2O, once with saturated brine, dried over sodium sulfate, and concentrated to a clear oil. The residue was purified by flash chromatography to afford the title compound (192 mg, 74%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.21 (s, 1H), 7.31 (d, *J* = 8.2 Hz, 2H), 7.16 (d, *J* = 8.3 Hz, 2H), 6.85 (s, 2H), 4.23 (s, 2H), 1.27 (dt, *J* = 6.1, 2.3 Hz, 4H). R_f = 0.26 (50% EtOAc/Hex).

Example 3.70: (*S*,*E*)-2,5-Dimethyl-*N*-(4-(1-(2,2,2-trifluoroacetamido)cyclopropyl)benzylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and Example 3.69 using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.56 (d, J = 8.4 Hz, 2H), 7.48 (t, J = 7.7 Hz, 2H), 7.37 (t, J = 7.4 Hz, 1H), 7.32 (d, J = 8.5 Hz, 2H), 7.28 (d, J = 8.5 Hz, 2H), 6.37 (d, J = 9.6 Hz, 1H), 5.07 (t, J = 10.0 Hz, 1H), 4.94 (s, 1H), 4.72 (s, 2H), 4.37 (s, 1H), 3.13 (s, 3H), 2.52 (s, 3H), 2.08-1.96 (m, 1H), 1.96 (d, J = 1.5 Hz, 3H), 1.49 (s, 3H), 1.40 (s, 3H), 1.35-1.27 (m, 4H), 1.10 (s, 9H), 0.92 (d, J = 7.1 Hz, 3H), 0.89 (d, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 170.93, 168.81, 165.64, 143.58, 142.24, 136.87, 134.19, 130.64, 129.00, 127.63, 127.53, 125.95, 125.61, 69.90, 57.10, 57.02, 56.39, 40.73, 34.55, 34.25, 32.80, 30.60, 29.33, 28.39, 25.57, 20.11, 18.38, 18.34, 16.21, 16.15, 14.04, 12.85. $C_{39}H_{54}F_3N_5O_6S$ calcd. m/z = 777.37 found $[M+H]^+$ = 778.55.

Example 3.71: (*S*,*E*)-*N*-(4-(1-Aminocyclopropyl)benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and Example 3.69 using General Procedures 9, 10 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.56 (d, J = 8.7 Hz, 2H), 7.51 (s, 4H), 7.47 (t, J = 7.6 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 6.49 (d, J = 9.5 Hz, 1H), 5.07 (t, J = 10.0 Hz, 1H), 4.94 (s, 1H), 4.81 (d, J = 14.0 Hz, 1H), 4.77 (d, J = 13.8 Hz, 1H), 4.39 (s, 1H), 3.16 (s, 3H), 2.52 (s, 3H), 2.11-1.99 (m, 1H), 1.97 (d, J = 1.5 Hz, 3H), 1.49 (s, 8H), 1.45-1.41 (m, 2H), 1.40 (s, 3H), 1.34-1.26 (m, 2H), 1.10 (s, 9H), 0.93 (d, J = 6.2 Hz, 3H), 0.90 (d, J = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 170.94, 169.00, 165.69, 143.57, 137.54, 137.12, 134.38, 131.43, 129.66, 128.98, 127.51, 125.98, 69.85, 65.51, 57.68, 57.15, 56.39, 40.72, 36.16, 34.51, 32.80, 30.68, 29.42, 28.40, 25.61, 20.14, 18.42, 18.39, 14.05, 12.86, 11.80. $C_{37}H_{55}N_5O_5S$ calcd. m/z = 681.39 found $[M+H]^+$ = 682.49.

Example 3.72: 1-Phenylcyclopropanamine.

The title compound was prepared as described in Bertus, P., Szymoniak, J. J. Org. Chem., 2003, 68, 7133-7136 from benzonitrile (1.0 mL, 9.7 mmol) to give 270 mg (21%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.44-7.28 (m, 4H), 7.27-7.15 (m, 1H), 1.18-1.06 (m, 2H), 1.07-0.95 (m, 2H). R_f = 0.28 (5% (5% NH_4OH/MeOH)/CH_2Cl_2).

Example 3.73: 2,2,2-Trifluoro-*N*-(1-phenylcyclopropyl)acetamide.

To a stirred solution of 1-phenylcyclopropanamine (270 mg, 2.03 mmol, 1.0 eq) in dioxane (5 mL), was added trifluoroacetic anhydride (0.310 mL, 2.23 mmol, 1.1 eq). After 5 min, TLC indicated complete conversion of starting material. The mixture was concentrated, then coevaporated once with

CH_2Cl_2 and once with toluene to yield the title compound (453 mg, 97%) as a flaky white powder. ^1H NMR (400 MHz, Chloroform-*d*) δ 7.47-7.15 (m, 5H), 6.88 (s, 1H), 1.65 (s, 4H). *m/z* calcd. for $C_11H_{10}F_3NO$ = 229.07. Found $[M+H]^+$ = 230.14. R_f = 0.82 (5% (5% NH_4OH/MeOH)/CH_2Cl_2).

Example 3.74: 2,2,2-Trifluoro-*N*-(1-(4-sulfamoylphenyl)cyclopropyl)acetamide.

To stirred chlorosulfonic acid (0.78 mL, 11.8 mmol, 6.0 eq) at 0 °C, was added solid 2,2,2-trifluoro-*N*-(1-phenylcyclopropyl)acetamide (450 mg, 1.96 mmol, 1.0 eq) portionwise, keeping the temperature low. After complete addition, the mixture was heated to 50 °C. After 10 minutes, gas evolution ceased, and the reaction was allowed to cool. The mixture was added slowly to a beaker of ice, being mindful of splattering. The solid that was left in the ice was filtered off. This solid was dried *in vacuo* and then taken up in THF (4 mL). Concentrated NH_4OH (0.44 mL, 7.85 mmol, 4.0 eq) was added, turning the solution green-black. After 2 min, TLC indicated complete consumption of the sulfonylchloride intermediate. 2M HCl was added until the color faded, then the mixture was extracted three times with EtOAc, washed once with saturated $NaHCO_3$, once with saturated brine, dried over sodium sulfate, and concentrated to a flaky solid. The crude material was purified by flash chromatography to yield the title compound (235 mg, 39%) as a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ 10.28 (s, 1H), 7.76 (d, *J* = 8.5 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 2H), 7.31 (s, 2H), 1.42-1.35 (m, 2H), 1.35-1.27 (m, 2H). *m/z* calcd. for $C_{11}H_{11}F_3N_2O_3S$ = 308.04. Found $[M+H]^+$ = 309.07. R_f = 0.27 (50% EtOAc/Hex).

Example 3.75: (*S*,*E*)-2,5-dimethyl-*N*-(4-(1-(2,2,2-trifluoroacetamido)cyclopropyl) phenylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and Example 3.3.74 using General Procedures 9 and 12. ^1H NMR (400 MHz, Methanol-d_4) δ 8.00 (d, *J* = 8.6 Hz, 2H), 7.55 (d, *J* = 7.6 Hz, 2H), 7.48 (t, *J* = 7.7 Hz, 2H), 7.48-7.33 (m, 4H), 6.47 (dd, *J* = 9.4, 1.6 Hz, 1H), 5.00 (t, *J* = 10.0 Hz, 1H), 4.92 (s, 1H), 4.35 (s, 1H), 3.15 (s, 3H), 2.51 (s, 3H), 2.11-2.00 (m, 1H), 1.86 (d, *J* = 1.4 Hz, 3H), 1.47 (d, *J* = 6.2 Hz, 3H), 1.45 (s, 2H), 1.43 (s, 2H), 1.38 (s, 3H), 1.06 (s, 9H), 0.91 (d, *J* = 6.1 Hz, 3H), 0.89 (d, *J* = 6.2 Hz, 3H). $C_{37}H_{50}F_3N_5O_6S$ calcd. *m/z* = 763.36 found $[M+H]^+$ = 764.45.

Example 3.76: (*S*,*E*)-*N*-(4-(1-Aminocyclopropyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2,2,2-trifluoro-*N*-(1-(4-sulfamoylphenyl)cyclopropyl)acetamide using General Procedures 9, 10 and 12. ^1H NMR (400 MHz, Methanol-d_4) δ 8.13 (d, *J* = 8.5 Hz, 2H), 7.66 (d, *J* = 8.6 Hz, 2H), 7.55 (d, *J* = 7.2 Hz, 2H), 7.47 (t, *J* = 7.6 Hz, 2H), 7.37 (t, *J* = 7.2 Hz, 1H), 6.50 (dd, *J* = 9.4, 1.7 Hz, 1H), 5.02 (t, *J* = 10.0 Hz, 1H), 4.93 (d,

J = 4.9 Hz, 1H), 4.38 (s, 1H), 3.16 (s, 3H), 2.51 (s, 3H), 2.12-1.99 (m, 1H), 1.84 (d, J = 1.4 Hz, 3H), 1.51-1.46 (m, 5H), 1.46-1.42 (m, 2H), 1.38 (s, 3H), 1.07 (s, 9H), 0.91 (dd, J = 6.7, 1.7 Hz, 6H). $C_{36}H_{53}N_5O_5S$ calcd. m/z = 667.38 found $[M+H]^+$ = 668.40.

Example 3.77: (*S*,*E*)-2,5-Dimethyl-*N*-(2-methylbenzylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2-methylbenzylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.61-7.52 (m, 2H), 7.48 (t, J = 7.6 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 7.30-7.23 (m, 3H), 7.22-7.14 (m, 1H), 6.48 (dd, J = 9.3, 1.7 Hz, 1H), 5.08 (t, J = 10.0 Hz, 1H), 4.94 (s, 1H), 4.81 (s, 2H), 4.34 (s, 1H), 3.15 (s, 3H), 2.51 (s, 3H), 2.48 (s, 3H), 2.08-2.00 (m, 1H), 1.98 (d, J = 1.1 Hz, 3H), 1.49 (s, 3H), 1.40 (s, 3H), 1.10 (s, 9H), 0.93 (d, J = 6.6 Hz, 3H), 0.91 (d, J = 6.6 Hz, 3H). $C_{35}H_{52}N_4O_5S$ calcd. m/z = 640.37 found $[M+H]^+$ = 641.41.

Example 3.78: (*S*,*E*)-2,5-Dimethyl-*N*-(4-nitrobenzylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 4-nitrobenzylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.18 (d, J = 8.7 Hz, 2H), 7.64 (d, J = 8.7 Hz, 2H), 7.52 (d, J = 7.5 Hz, 2H), 7.42 (t, J = 7.7 Hz, 2H), 7.31 (t, J = 7.3 Hz, 1H), 6.55 (d, J = 9.4 Hz, 1H), 5.04 (t, J = 10.0 Hz, 1H), 4.92 (s, 1H), 4.63 (s, 2H), 3.08 (s, 3H), 2.32 (s, 3H), 1.95 (dt, J = 11.4, 6.6 Hz, 4H), 1.89 (d, J = 1.4 Hz, 3H), 1.46 (s, 3H), 1.38 (s, 3H), 1.05 (s, 9H), 0.89 (d, J = 6.5 Hz, 3H), 0.85 (d, J = 6.5 Hz, 3H). $C_{34}H_{49}N_5O_7S$ calcd. m/z = 671.34 found $[M+H]^+$ = 672.36.

Example 3.79: (*S*,*E*)-*N*-(4-Chlorobenzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 4-chlorobenzylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.56 (d, J = 7.9 Hz, 2H), 7.48 (t, J = 7.6 Hz, 2H), 7.44-7.34 (m, 5H), 6.39 (d, J = 9.5 Hz, 1H), 5.06 (t, J = 10.0 Hz, 1H), 4.94 (s, 1H), 4.75 (s, 2H), 4.35 (s, 1H), 3.13 (s, 3H), 2.51 (s, 3H), 2.06-1.95 (m, 1H), 1.95 (d, J = 1.4 Hz, 3H), 1.49 (s, 3H), 1.39 (s, 3H), 1.09 (s, 9H), 0.91 (d, J = 6.1 Hz, 3H), 0.89 (d, J = 5.9 Hz, 3H). $C_{34}H_{49}ClN_4O_5S$ calcd. m/z = 660.31 found $[M+H]^+$ = 661.32.

Example 3.80: (*S*,*E*)-2,5-Dimethyl-*N*-(phenethylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and homobenzylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.56 (d, J = 7.6 Hz, 2H), 7.48 (t, J = 7.5 Hz, 2H), 7.38 (t, J = 7.4 Hz, 1H), 7.34-7.28 (m, 2H), 7.28-7.20 (m, 3H), 6.47 (dd, J = 9.2, 1.7 Hz, 1H), 5.03 (t, J = 10.0 Hz, 1H), 4.94 (s, 1H), 4.36 (d, J = 2.3 Hz, 2H), 3.78 (td, J = 7.5, 4.1 Hz, 2H), 3.17 (s,

3H), 3.12 (t, J = 7.8 Hz, 2H), 2.51 (s, 3H), 2.14-2.01 (m, 1H), 1.89 (d, J = 1.4 Hz, 3H), 1.49 (s, 3H), 1.39 (s, 3H), 1.09 (s, 9H), 0.94 (d, J = 6.6 Hz, 3H), 0.91 (d, J = 6.6 Hz, 3H). $C_{35}H_{52}N_4O_5S$ calcd. *m/z* = 640.37 found $[M+H]^+$ = 641.36.

Example 3.81: (*S*,*E*)-*N*-(4-Bromobenzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 4-bromobenzylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.60-7.51 (m, 4H), 7.48 (t, J = 7.7 Hz, 2H), 7.39 (s, 1H), 7.31 (d, J = 8.3 Hz, 2H), 6.38 (d, J = 9.3 Hz, 1H), 5.06 (t, J = 10.0 Hz, 1H), 4.93 (s, 1H), 4.74 (s, 2H), 4.36 (s, 1H), 3.13 (s, 3H), 2.52 (s, 3H), 2.03-1.98 (m, 1H), 1.95 (d, J = 1.4 Hz, 3H), 1.49 (s, 3H), 1.39 (s, 3H), 1.09 (s, 9H), 0.91 (d, J = 6.1 Hz, 3H), 0.89 (d, J = 6.3 Hz, 3H) $C_{34}H_{49}BrN_4O_5S$ calcd. *m/z* = 704.26 found $[M+H]^+$ = 705.23.

Example 3.82: (*S*,*E*)-*N*-(4-Cyanobenzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 4-cyanobenzylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.77 (d, J = 8.3 Hz, 2H), 7.64-7.53 (m, 4H), 7.48 (t, J = 7.7 Hz, 2H), 7.38 (t, J = 7.3 Hz, 1H), 6.41 (dd, J = 9.3, 1.7 Hz, 1H), 5.05 (t, J = 10.0 Hz, 1H), 4.94 (s, 1H), 4.87 (s, 2H), 4.36 (s, 1H), 3.14 (s, 3H), 2.52 (s, 3H), 2.06-1.98 (m, 1H), 1.95 (d, J = 1.4 Hz, 3H), 1.49 (s, 3H), 1.39 (s, 3H), 1.09 (s, 9H), 0.91 (d, J = 4.0 Hz, 3H), 0.90 (d, J = 4.0 Hz, 3H). $C_{35}H_{49}N_5O_5S$ calcd. *m/z* = 651.35 found $[M+H]^+$ = 652.38.

Example 3.83: (*S*,*E*)-2,5-Dimethyl-*N*-(3-nitrobenzylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 3-nitrobenzylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.29 (d, J = 8.0 Hz, 1H), 8.26 (s, 1H), 7.83 (d, J = 7.8 Hz, 1H), 7.67 (t, J = 8.0 Hz, 1H), 7.56 (d, J = 7.2 Hz, 2H), 7.48 (t, J = 7.7 Hz, 2H), 7.38 (t, J = 7.3 Hz, 1H), 6.43 (dd, J = 9.4, 1.7 Hz, 1H), 5.05 (t, J = 10.0 Hz, 1H), 4.93 (s, 2H), 4.93 (s, 1H), 4.36 (s, 1H), 3.13 (s, 3H), 2.52 (s, 3H), 2.08-1.98 (m, 1H), 1.96 (d, J = 1.4 Hz, 3H), 1.48 (s, 3H), 1.39 (s, 3H), 1.07 (s, 9H), 0.89 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H). $C_{34}H_{49}N_5O_7S$ calcd. *m/z* = 671.34 found $[M+H]^+$ = 672.39.

Example 3.84: (*S*,*E*)-*N*-(4-*tert*-Butylbenzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 4-*t*-butylbenzylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.56 (d, J = 7.6 Hz, 2H), 7.48 (t, J

= 7.7 Hz, 2H), 7.43 (d, *J* = 8.2 Hz, 2H), 7.38 (t, *J* = 7.3 Hz, 1H), 7.30 (d, *J* = 8.2 Hz, 2H), 6.39 (dd, *J* = 9.4, 1.6 Hz, 1H), 5.07 (t, *J* = 10.0 Hz, 1H), 4.93 (s, 1H), 4.72 (s, 2H), 4.37 (s, 1H), 3.13 (s, 3H), 2.52 (s, 3H), 2.06-1.98 (m, 1H), 1.96 (d, *J* = 1.4 Hz, 3H), 1.49 (s, 3H), 1.39 (s, 3H), 1.33 (s, 9H), 1.10 (s, 9H), 0.92 (d, *J* = 6.6 Hz, 3H), 0.89 (d, *J* = 6.5 Hz, 3H). $C_{38}H_{58}N_4O_5S$ calcd. *m/z* = 682.41 found $[M+H]^+$ = 683.47.

Example 3.85: (*S*,*E*)-2,5-Dimethyl-*N*-(2-nitrobenzylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2-nitrobenzylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.03 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.72 (td, *J* = 7.5, 1.5 Hz, 1H), 7.65 (td, *J* = 7.7, 1.6 Hz, 1H), 7.60 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.56 (d, *J* = 7.2 Hz, 2H), 7.48 (t, *J* = 7.7 Hz, 2H), 7.38 (t, *J* = 7.3 Hz, 1H), 6.43 (dd, *J* = 9.4, 1.6 Hz, 1H), 5.31 (d, *J* = 14.2 Hz, 1H), 5.26 (d, *J* = 15.3 Hz, 1H), 5.06 (t, *J* = 10.0 Hz, 1H), 4.94 (s, 1H), 4.37 (s, 1H), 3.15 (s, 3H), 2.52 (s, 3H), 2.08-1.98 (m, 1H), 1.96 (d, *J* = 1.4 Hz, 3H), 1.49 (s, 3H), 1.39 (s, 3H), 1.10 (s, 9H), 0.92 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 3H). $C_{34}H_{49}N_5O_7S$ calcd. *m/z* = 671.34 found $[M+H]^+$ = 672.39.

Example 3.86: (*S*,*E*)-2,5-Dimethyl-*N*-(4-nitrophenethylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 4-nitro-homobenzylsulfonamide using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.19 (d, *J* = 8.7 Hz, 2H), 7.58-7.51 (m, 4H), 7.47 (t, *J* = 7.6 Hz, 2H), 7.37 (t, *J* = 7.3 Hz, 1H), 6.47 (dd, *J* = 9.5, 1.7 Hz, 1H), 5.00 (t, *J* = 10.0 Hz, 1H), 4.93 (s, 1H), 4.36 (s, 1H), 3.91 (dd, *J* = 14.9, 8.5 Hz, 1H), 3.84 (dd, *J* = 12.9, 8.5 Hz, 1H), 3.28 (t, *J* = 7.5 Hz, 2H), 3.16 (s, 3H), 2.51 (s, 3H), 2.12-1.98 (m, 1H), 1.87 (d, *J* = 1.4 Hz, 3H), 1.48 (s, 3H), 1.39 (s, 3H), 1.08 (s, 9H), 0.91 (d, *J* = 6.6 Hz, 3H), 0.91 (d, *J* = 6.6 Hz, 3H). $C_{35}H_{51}N_5O_7S$ calcd. *m/z* = 685.35 found $[M+H]^+$ = 686.38.

Example 3.87: Methyl 4-Chloro-3-(*N*-((*S*,*E*)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enoyl)sulfamoyl)benzoate.

Title compound was prepared from Example 3.16 and methyl 4-chloro-3-sulfamoylbenzoate using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.80 (d, *J* = 2.1 Hz, 1H), 8.20 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.71 (d, *J* = 8.3 Hz, 1H), 7.59-7.52 (m, 2H), 7.47 (t, *J* = 7.7 Hz, 2H), 7.40-7.32 (m, 1H), 6.63-6.56 (m, 1H), 5.02 (t, *J* = 10.0 Hz, 1H), 4.37 (s, 1H), 3.98 (s, 3H), 3.18 (s, 3H), 2.51 (s, 3H), 2.13-2.00 (m, 1H), 1.86 (d, *J* = 1.4 Hz, 3H), 1.47 (s, 3H), 1.37 (s, 3H), 1.06 (s, 9H), 0.96-0.87 (m, 6H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 170.87, 165.65, 164.87, 143.61, 137.01, 136.04, 134.29, 133.23, 131.81, 129.16, 128.98, 128.88, 127.50, 125.98, 69.81, 65.53, 57.39, 56.35, 56.15,

55.37, 51.86, 40.70, 34.51, 32.77, 30.80, 29.39, 28.44, 26.18, 25.56, 20.06, 18.40, 14.06, 12.74. $C_{35}H_{49}ClN_4O_7S$ calcd. *m/z* = 704.30 amu; found $[M+H]^+$ = 705.25, [M+Na]+ = 727.25.

Example 3.88: 2,2,2-Trifluoro-*N*-(4-(sulfamoylmethyl)benzyl)acetamide.

The title compound was synthesized from commercially available (4-(aminomethyl)phenyl)methanesulfonamide and TFAA using General Procedure 8. ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.05 (s, 1H), 7.48-7.40 (m, 2H), 7.40-7.32 (m, 2H), 6.17 (s, 1H), 4.56 (d, *J* = 6.1 Hz, 2H), 4.35 (s, 2H).

Example 3.89: (*S*,*E*)-2,5-Dimethyl-*N*-(4-((2,2,2-trifluoroacetamido)methyl)benzylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and Example 3.88 using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.57-7.49 (m, 2H), 7.45 (t, *J* = 7.5 Hz, 2H), 7.33 (p, *J* = 8.8, 7.9 Hz, 5H), 6.37 (d, *J* = 9.7 Hz, 1H), 5.09-5.00 (m, 1H), 4.69 (s, 2H), 4.44 (s, 2H), 4.30 (s, 1H), 3.10 (s, 3H), 2.45 (d, *J* = 17.5 Hz, 3H), 2.02-1.87 (m, 4H), 1.46 (s, 3H), 1.37 (s, 3H), 1.07 (s, 9H), 0.95-0.81 (m, 6H). ^{19}F NMR (377 MHz, Methanol-d_4) δ-76.94,-77.24. $C_{37}H_{52}F_3N_5O_6S$ calcd. *m/z* = 751.36 amu; found $[M+H]^+$ = 752.46, [M+Na]+ = 774.38.

Example 3.90: (*S*,*E*)-*N*-(4-(Aminomethyl)benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Prepared from Example 3.16 and Example 3.88 using General Procedures 9, 10 and 12 ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.60-7.54 (m, 2H), 7.54-7.50 (m, 4H), 7.47 (d, *J* = 8.1 Hz, 2H), 7.37 (t, *J* = 7.4 Hz, 1H), 6.49 (dd, *J* = 9.5, 1.5 Hz, 1H), 5.07 (t, *J* = 10.0 Hz, 1H), 4.94 (s, 1H), 4.83 (d, *J* = 14.3 Hz, 1H), 4.79 (d, *J* = 13.9 Hz, 1H), 4.38 (s, 1H), 4.16 (s, 2H), 3.16 (s, 3H), 2.52 (s, 3H), 2.10-2.00 (m, 1H), 1.97 (d, *J* = 1.4 Hz, 3H), 1.49 (s, 3H), 1.40 (s, 3H), 1.10 (s, 9H), 0.93 (d, *J* = 6.9 Hz, 3H), 0.91 (d, *J* = 7.0 Hz, 3H). $C_{35}H_{53}N_5O_5S$ calcd. *m/z* = 655.4; found $[M+H]^+$ = 656.3, [M+2H]2+ = 328.8.

Example 3.91: 2,2,2-Trifluoro-*N*-(4-(sulfamoylmethyl)phenyl)acetamide.

The title compound was synthesized from commercially available (4-aminophenyl)methanesulfonamide and TFAA using General Procedure 8. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H), 7.79-7.51 (m, 2H), 7.51-7.23 (m, 2H), 6.85 (s, 2H), 4.27 (s, 2H).

Example 3.92: (*S*,*E*)-2,5-Dimethyl-*N*-(4-(2,2,2-trifluoroacetamido)benzylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and Example 3.91 using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.68 (d, J = 8.6 Hz, 2H), 7.54 (d, J = 7.1 Hz, 2H), 7.45 (t, J = 7.6 Hz, 2H), 7.37 (dd, J = 10.6, 5.0 Hz, 3H), 6.34 (d, J = 9.4 Hz, 1H), 5.04 (t, J = 10.1 Hz, 2H), 4.74 (s, 2H), 4.35 (s, 1H), 3.10 (s, 3H), 2.49 (s, 3H), 2.02-1.94 (m, 1H), 1.93 (d, J = 1.4 Hz, 3H), 1.46 (s, 3H), 1.37 (s, 3H), 1.06 (s, 9H), 0.88 (d, J = 6.3 Hz, 3H), 0.86 (s, 3H). ^{19}F NMR (377 MHz, Methanol-d_4) δ-76.97,-77.05. $C_{36}H_{50}F_3N_5O_6S$ calcd. m/z = 737.34 amu; found $[M+H]^+$ = 738.38, [M+Na]+ = 760.35.

Example 3.93: (*S*,*E*)-*N*-(4-Aminobenzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and Example 3.91 using General Procedures 9, 10 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.56 (d, J = 7.6 Hz, 2H), 7.48 (t, J = 7.7 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 7.20 (d, J = 8.5 Hz, 2H), 6.87 (d, J = 8.5 Hz, 2H), 6.39 (d, J = 9.4 Hz, 1H), 5.07 (t, J = 10.0 Hz, 1H), 4.95 (s, 1H), 4.64 (s, 2H), 4.38 (s, 1H), 3.14 (s, 3H), 2.52 (s, 3H), 2.07-1.98 (m, 1H), 1.96 (d, J = 1.4 Hz, 3H), 1.49 (s, 3H), 1.39 (s, 3H), 1.10 (s, 9H), 0.92 (d, J = 6.7 Hz, 3H), 0.90 (d, J = 6.4 Hz, 3H). $C_{34}H_{51}N_5O_5S$ calcd. m/z = 641.4; found $[M+H]^+$ = 642.3.

Example 3.94: 4-(Azidomethyl)benzenesulfonamide.

To a stirred solution of 4-(bromomethyl)benzenesulfonamide (0.50 g) in *N*,*N*-dimethylformamide (1mL) was added sodium azide (0.20 g). The suspension was heated to 50 °C for 3 hours at which points the solvent was removed under reduced pressure. The residue was partitioned between ethyl acetate and water. The organic phase was washed with brine, dried over magnesium sulfate, filtered and concentrated to dryness to give the title compound as a syrup that solidified on standing. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.06-7.91 (m, 2H), 7.58-7.44 (m, 2H), 4.96 (s, 2H), 4.48 (s, 2H).

Example 3.95: 4-(Aminomethyl)benzenesulfonamide

To a solution of 4-(azidomethyl)benzenesulfonamide (0.354g) in methanol (10 mL) in a round bottom flask equipped with a magnetic stirrer was added 10% Pd/C (~0.05g). The flask was evacuated of gases at reduced pressure and charged with hydrogen. This evacuation and charge was repeated three times at which point the suspension was left to stir overnight. At 16h, TLC analysis indicated complete consumption of the starting material. The reaction was diluted with methanol (40 mL), Celite was added and the mixture was filtered through a fritted glass funnel. The resulting solution was concentrated to dryness. ^{1}H NMR suggested that the material was sufficiently clean at this stage for further use without purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.77 (m, 2H), 7.53 (m, 2H), 5.76 (s, 2H), 3.76 (d, J = 11.9 Hz, 2H).

Example 3.96: 2,2,2-Trifluoro-*N*-(4-sulfamoylbenzyl)acetamide.

The title compound was synthesized by reaction of 4-(aminomethyl)benzenesulfonamide with TFAA according to General Procedure 8, with a ^{1}H NMR spectrum that was complicated by rotamers. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91-7.75 (m, 2H), 7.55-7.31 (m, 4H), 4.72 (m, 2H), 4.47 (d, J = 6.0 Hz, 1H), 3.18 (s, 2H).

Example 3.97: (*S*,*E*)-2,5-Dimethyl-*N*-(4-((2,2,2-trifluoroacetamido)methyl)phenylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and Example 3.96 using General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.02 (d, J = 8.5 Hz, 2H), 7.58-7.42 (m, 7H), 7.35 (t, J = 7.3 Hz, 1H), 6.46 (d, J = 8.5 Hz, 1H), 4.97 (d, J = 10.4 Hz, 1H), 4.54 (s, 2H), 4.33 (s, 1H), 3.14 (s, 3H), 2.48 (s, 3H), 2.11-1.97 (m, 1H), 1.83 (d, J = 1.4 Hz, 3H), 1.53 (s, 1H), 1.44 (s, 3H), 1.34 (s, 3H), 1.04 (s, 9H), 0.89 (d, J = 3.9 Hz, 3H), 0.88 (d, J = 4.1 Hz, 3H). ^{19}F NMR (377 MHz, Methanol-d_4) δ-76.94,-77.26. $C_{36}H_{50}F_3N_5O_6S$ calcd. m/z = 737.34 amu; found $[M+H]^+$ = 738.39, [M+Na]+ = 760.41

Example 3.98: (*S*,*E*)-*N*-(4-(Aminomethyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Prepared from Example 3.16 and Example 3.96 using General Procedures 9, 10 and 12 ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.13 (d, J = 8.3 Hz, 2H), 7.68 (d, J = 8.3 Hz, 2H), 7.55 (d, J = 7.6 Hz, 2H), 7.47 (t, J = 7.7 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 6.51 (dd, J = 9.2, 1.8 Hz, 1H), 5.01 (t, J = 10.0 Hz, 1H), 4.37 (s, 1H), 4.24 (s, 2H), 3.17 (s, 3H), 2.51 (s, 3H), 2.13-1.97 (m, 1H), 1.84 (d, J = 1.4 Hz, 3H), 1.47 (s, 3H), 1.37 (s, 3H), 1.07 (s, 9H), 0.91 (dd, J = 6.7, 2.0 Hz, 7H). $C_{34}H_{51}N_5OS$ calcd. m/z = 641.36 amu; found $[M+H]^+$ = 642.4.

Example 3.99: (*S*,*E*)-*N*-(Benzylsulfonyl)-4-((*S*)-2-((*S*)-3-(4-bromophenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enamide.

Title compound was prepared from Example 3.51 and (*S*,*E*)-4-((*S*)-2-amino-*N*,3,3-trimethylbutanamido)-*N*-(benzylsulfonyl)-2,5-dimethylhex-2-enamide using General Procedures 11 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.62 (t, J = 9.2 Hz, 2H), 7.50-7.43 (m, 2H), 7.38 (d, J = 2.2 Hz, 5H), 6.38 (dd, J = 9.5, 1.8 Hz, 1H), 5.05 (t, J = 10.0 Hz, 1H), 4.92 (s, 1H), 4.75 (d, J = 2.2 Hz, 2H), 4.30 (s, 1H), 3.12 (s, 3H), 2.53 (s, 3H), 2.06-1.97 (m, 1H), 1.95 (d, J = 1.5 Hz, 3H), 1.47 (s, 3H), 1.39 (s, 3H), 1.09 (s, 9H), 0.94-0.86 (m, 6H). $C_{34}H_{49}BrN_4O_5S$ calcd. m/z = 704.26 amu; found $[M+H]^+$ = 705.29, $[M+Na]^+$ = 727.36.

Example 3.100: (*S*,*E*)-4-((*S*)-2-((*S*)-3-(4'-Acetylbiphenyl-4-yl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-*N*-(benzylsulfonyl)-2,5-dimethylhex-2-enamide.

Title compound was prepared according to General Procedure 13 from Boc protected Example 3.99 and 4-acetylphenylboronic acid. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.15-8.08 (m, 2H), 7.86-7.76 (m, 4H), 7.66 (dd, J = 14.7, 8.4 Hz, 2H), 7.38 (d, J = 4.9 Hz, 5H), 6.39 (d, J = 9.3 Hz, 1H), 5.05 (t, J = 10.1 Hz, 1H), 4.94 (s, 1H), 4.75 (d, J = 4.1 Hz, 2H), 4.37 (d, J = 16.1 Hz, 1H), 3.13 (d, J = 3.4 Hz, 3H), 2.67 (s, 3H), 2.53 (d, J = 11.6 Hz, 3H), 2.01 (s, 1H), 1.96 (d, J = 1.5 Hz, 3H), 1.54 (d, J = 3.7 Hz, 3H), 1.44 (s, 3H), 1.09 (d, J = 2.7 Hz, 9H), 0.96-0.83 (m, 6H). $C_{42}H_{56}N_4O_6S$ calcd. *m/z* = 744.39 amu; found $[M+H]^+$ = 745.42, $[M+Na]^+$ = 767.36.

Example 3.101: (*S*,*E*)-*N*-(Benzylsulfonyl)-4-((*S*)-2-((*S*)-3-(4'-methoxybiphenyl-4-yl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enamide.

Title compound was prepared according to General Procedure 13 from Boc protected Example 3.99 and 4-methoxyphenylboronic acid. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.74-7.53 (m, 6H), 7.38 (d, J = 4.7 Hz, 5H), 7.08-6.99 (m, 2H), 6.43-6.35 (m, 1H), 5.06 (s, 1H), 4.94 (s, 1H), 4.75 (d, J = 4.1 Hz, 2H), 4.38 (s, 1H), 3.86 (s, 3H), 3.13 (s, 3H), 2.54 (s, 3H), 1.99 (d, J = 11.0 Hz, 1H), 1.96 (d, J = 1.5 Hz, 3H), 1.51 (s, 3H), 1.43 (s, 3H), 1.09 (s, 9H), 0.96-0.85 (m, J = 6.0, 5.1 Hz, 6H). $C_{41}H_{56}N_4O_6S$ calcd. *m/z* = 732.39 amu; found $[M+H]^+$ = 733.41, $[M+Na]^+$ = 755.40.

Example 3.102: (*S*,*E*)-*N*-(Benzylsulfonyl)-4-((*S*)-2-((*S*)-3-(biphenyl-4-yl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enamide.

Title compound was prepared according to General Procedure 13 from Boc protected Example 3.99 and phenylboronic acid. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.86-7.51 (m, 6H), 7.48 (t, J = 7.6 Hz, 2H), 7.43-7.33 (m, 6H), 6.39 (d, J = 9.5 Hz, 1H), 5.06 (t, J = 10.1 Hz, 1H), 4.94 (s, 1H), 4.75 (d, J = 3.3 Hz, 2H), 4.37 (d, J = 14.4 Hz, 1H), 3.13 (d, J = 3.7 Hz, 3H), 2.55 (d, J = 4.5 Hz, 3H), 2.06-1.97 (m, 1H), 1.96 (d, J = 1.5 Hz, 3H), 1.52 (s, 3H), 1.44 (d, J = 4.5 Hz, 3H), 1.09 (d, J = 5.6 Hz, 9H), 0.96-0.83 (m, 6H). $C_{40}H_{54}N_4O_5S$ calcd. *m/z* = 702.38 amu; found $[M+H]^+$ = 703.40, [M+Na]+ = 725.45.

Example 3.103: (*S*,*E*)-*N*-(Benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-(4-(4-methylstyryl)phenyl)butanamido)butanamido)hex-2-enamide.

Title compound was prepared according to General Procedure 13 from Boc protected Example 3.99 and (*E*)-4-methylstyrylboronic acid. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.65 (d, J = 8.2 Hz, 2H), 7.54 (d, J = 8.2 Hz, 2H), 7.47 (d, J = 7.8 Hz, 2H), 7.38 (s, 5H), 7.26-7.11 (m, 4H), 6.39 (d, J = 9.3 Hz, 1H), 5.06 (t, J = 10.0 Hz, 1H), 4.97-4.91 (m, 1H), 4.76 (s, 2H), 4.36 (s, 1H), 3.12 (d, J

= 8.9 Hz, 3H), 2.54 (s, 3H), 2.37 (s, 3H), 2.05-1.97 (m, 1H), 1.97-1.93 (m, 3H), 1.49 (s, 3H), 1.41 (s, 3H), 1.09 (d, J = 3.5 Hz, 9H), 0.91 (tq, J = 10.8, 4.9 Hz, 6H). $C_{43}H_{58}N_4O_5S$ calcd. m/z = 742.41 amu; found $[M+H]^+$ = 743.44, [M+Na]+ = 765.41.

Example 3.104: (*S*,*E*)-*N*-(Benzylsulfonyl)-4-((*S*)-2-((*S*)-3-(4-methoxyphenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enamide.

Title compound was prepared according to General Procedure 14 from Boc protected Example 3.99. Major diastereomer: ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.44 (dd, J = 12.9, 8.6 Hz, 2H), 7.40-7.34 (m, 5H), 7.00 (t, J = 8.4 Hz, 2H), 6.38 (d, J = 9.2 Hz, 1H), 5.05 (t, J = 9.9 Hz, 1H), 4.93 (s, 1H), 4.75 (d, J = 1.8 Hz, 2H), 4.29 (s, 1H), 3.84 (s, 3H), 3.12 (s, 3H), 2.51 (s, 3H), 2.04-1.98 (m, 1H), 1.95 (d, J = 1.4 Hz, 3H), 1.45 (s, 3H), 1.37 (s, 3H), 1.09 (s, 9H), 0.92-0.86 (m, 6H). Minor diastereomer: ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.44 (dd, J = 12.9, 8.6 Hz, 2H), 7.40-7.34 (m, 5H), 7.00 (t, J = 8.4 Hz, 2H), 6.38 (d, J = 9.2 Hz, 1H), 4.99 (t, J = 10.1 Hz, 1H), 4.93 (s, 1H), 4.75 (d, J = 1.8 Hz, 2H), 4.26 (s, 1H), 3.82 (s, 3H), 3.11 (s, 3H), 2.47 (s, 3H), 2.04-1.98 (m, 1H), 1.92 (d, J = 1.4 Hz, 3H), 1.53 (s, 3H), 1.48 (s, 3H), 0.94 (s, 9H), 0.92-0.86 (m, 6H). $C_{35}H_{52}N_4O_6S$ calcd. m/z = 656.36 amu; found $[M+H]^+$ = 657.35, [M+Na]+ = 679.25.

Example 3.105: (*S*,*E*)-*N*-(Benzylsulfonyl)-4-((*S*)-2-((R)-3-(3-methoxyphenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enamide.

Title compound was prepared according to General Procedure 14 from Boc protected (*S*,*E*)-*N*-(benzylsulfonyl)-4-((*S*)-2-((*S*)-3-(3-bromophenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enamide. The two diastereomeric products resulted from diastereomerically impure starting material and were separable by prep-scale HPLC. Major diastereomer: ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.51-7.32 (m, 6H), 7.14-7.07 (m, 1H), 7.06 (t, J = 2.2 Hz, 1H), 6.98-6.90 (m, 1H), 6.38 (dd, J = 9.6, 1.7 Hz, 1H), 4.99 (t, J = 10.3 Hz, 1H), 4.93 (s, 1H), 4.75 (d, J = 1.8 Hz, 2H), 4.32 (s, 1H), 3.85 (s, 3H), 3.11 (s, 3H), 2.47 (s, 3H), 2.04-1.96 (m, 1H), 1.93 (d, J = 1.4 Hz, 3H), 1.54 (s, 3H), 1.47 (s, 3H), 0.96 (s, 9H), 0.89 (dd, J = 6.6, 3.4 Hz, 6H). Minor diastereomer: refer to Example 3.106 (immediately following) for ^{1}H NMR spectral data $C_{35}H_{52}N_4O_6S$ calcd. m/z = 656.36 amu; found $[M+H]^+$ = 657.36, [M+Na]+ = 679.29.

Example 3.106: (*S*,*E*)-*N*-(Benzylsulfonyl)-4-((*S*)-2-((*S*)-3-(3-methoxyphenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enamide.

Title compound was prepared according to Example 3.105. The two diastereomeric products resulted from diastereomerically impure starting material and were separable by prep-scale HPLC. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.39 (d, J = 5.5 Hz, 6H), 7.11 (dd, J = 4.9, 2.8 Hz, 3H), 6.38 (d, J = 9.4 Hz, 1H), 5.06 (d, J = 9.5 Hz, 1H), 4.93 (s, 1H), 4.76 (s, 2H), 4.35 (s, 1H), 3.86 (s, 3H), 3.13 (s,

3H), 2.52 (s, 3H), 2.05-1.97 (m, 1H), 1.95 (d, J = 1.6 Hz, 3H), 1.46 (s, 3H), 1.38 (s, 3H), 1.09 (s, 9H), 0.90 (t, J = 6.6 Hz, 6H). $C_{35}H_{52}N_4O_6S$ calcd. m/z = 656.36 amu; found $[M+H]^+$ = 657.36, [M+Na]+ = 679.32.

Example 3.107: (*S*,*E*)-*N*-(Benzylsulfonyl)-4-((*S*)-2-((*S*)-3-(4-(2-hydroxyethoxy)phenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enamide.

Title compound was prepared as follows: a mixture of Boc protected Example 3.99, CuI (10 mol%), 3,4,7,8-tetramethyl-1,10-phenanthroline (20 mol%), Cs_2CO_3 (2.5 eq), and ethylene glycol (90 eq) was stirred under N_2 at 130 °C for 20 h. The resulting mixture was diluted with H_2O, carefully acidified with 1M citric acid and extracted with CH_2Cl_2 (5×). The organics were combined, washed with brine (1×), dried over $MgSO_4$, filtered, concentrated *in vacuo* and purified via silica gel column chromatography (eluted with AcOH/EtOAc/hexanes mixtures) to afford the cross-coupled product which was subsequently deprotected and purified according to General Procedure 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.46 (d, J = 8.8 Hz, 2H), 7.38 (d, J = 2.5 Hz, 5H), 7.05 (d, J = 8.4 Hz, 2H), 6.38 (d, J = 9.5 Hz, 1H), 5.05 (t, J = 10.1 Hz, 1H), 4.93 (s, 1H), 4.76 (s, 2H), 4.28 (d, J = 11.0 Hz, 1H), 4.13-4.04 (m, 2H), 3.90 (t, J = 4.6 Hz, 2H), 3.12 (d, J = 6.2 Hz, 3H), 2.50 (d, J = 16.9 Hz, 3H), 2.05-1.97 (m, 1H), 1.94 (d, J = 11.0 Hz, 3H), 1.56-1.34 (m, 6H), 1.09 (s, 9H), 0.90 (t, J = 6.4 Hz, 6H). $C_{36}H_{54}N_4O_7S$ calcd. m/z = 686.37 amu; found $[M+H]^+$ = 687.42, [M+Na]+ = 709.37.

Example 3.108: *S*-2-(4-((*S*)-4-((*S*)-1-(((*S*,*E*)-2,5-Dimethyl-6-oxo-6-(benzylsulfonamido)hex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-ylamino)-2-methyl-3-(methylamino)-4-oxobutan-2-yl)phenoxy)ethyl ethanethioate.

Title compound was prepared as follows: Tributylphosphine (6 eq) was added to a cold (0 °C) stirring solution of di-*tert*-butyl azodicarboxylate (6 eq) in THF. After 0.5 h, a solution of the Boc protected Example 3.107 (1 eq) in THF was added, followed by a solution of AcSH (4.5 eq) in THF. The pale yellow mixture was stirred at 0 °C for 1 h then at ambient temperature for 23 h. The resulting mixture was concentrated *in vacuo*, dissolved in EtOAc and successively washed with 1M HCl (2×), sat'd NH_4Cl (1×) and brine (1×). The organics were dried over $MgSO_4$, filtered, concentrated *in vacuo* and purified via silica gel column chromatography (eluted with AcOH/EtOAc/hexanes mixtures) to afford the Boc-protected thioacetate product (HPLC/MS-$[M+Na]^+$ = 867.47). The thioacetate was dissolved in CH_2Cl_2 and treated with TFA. After stirring for 1 h, the reaction mixture was concentrated *in vacuo*. The yellow/brown residue was dissolved in minimal amount of CH_2Cl_2, cooled to 0 °C and treated with ether to precipitate out the desired aminothioacetate as an off-white solid in 10% yield over two synthetic steps. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.46 (d, J = 8.7 Hz, 2H), 7.38 (d, J = 2.4 Hz, 5H), 7.03 (d, J = 8.6 Hz, 2H), 6.38 (d, J = 9.5 Hz, 1H), 5.05 (t, J = 10.0 Hz, 1H), 4.93 (s, 1H), 4.75 (s, 2H), 4.27 (d, J = 11.4 Hz, 1H), 4.14 (t, J = 6.6 Hz, 2H), 3.28 (t, J = 6.6 Hz, 2H), 3.11

(d, J = 6.6 Hz, 3H), 2.49 (d, J = 15.5 Hz, 3H), 2.38 (s, 3H), 2.05-1.97 (m, 1H), 1.95 (s, 3H), 1.45 (s, 3H), 1.37 (s, 3H), 1.08 (s, 9H), 0.96-0.85 (m, 6H). $C_{38}H_{56}N_4O_7S_2$ calcd. m/z = 744.36 amu; found $[M+H]^+$ = 745.39, $[M+Na]^+$ = 777.32.

Example 3.109: (*S*,*E*)-4-((*S*)-2-((*S*)-3-(4-(2-Aminoethoxy)phenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-*N*-(benzylsulfonyl)-2,5-dimethylhex-2-enamide.

Title compound was prepared as follows: Et_3N (4 eq) was added to a cold (0 °C) stirring solution of MsCl (3.7 eq) in CH_2Cl_2. After 2 min, a solution of the Boc protected Example 3.107 in CH_2Cl_2 was added. The pale yellow mixture was stirred cold for 5 min and then at ambient temperature for 72 h. The resulting mixture was dilute with EtOAc and successively washed with 1M citric acid (1×), 1M $NaHCO_3$ (1×) and brine (1×). The organics were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the mesylated-alcohol (HPLC/MS-$[M+Na]^+$ = 887.42) which was used in the next step without further purification.

The mesylate was dissolved in DMF and treated with NaN_3 (7 eq). The resulting suspension was stirred at ambient temperature for 18 h and then at 60 °C for 5 h. The reaction mix was diluted with H_2O, acidified with 1M HCl and extracted with CH_2Cl_2 (4×). The combined organics were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the azido product (HPLC/MS-$[M+Na]^+$ = 834.44) which was used in the next step without further purification.

The azide was dissolved in THF/H_2O (10:1) and treated with tributylphosphine (3.5 eq). The mixture was stirred at ambient temperature for 21 h and then concentrated *in vacuo*. The resulting residue was dissolved in EtOAc and successively washed with 1M HCl (3×), 1M NaHCO3 (3×), H2O (2×) and brine (2×). The organics were dried over $MgSO_4$, filtered, concentrated *in vacuo* and purified via silica gel column chromatography (eluted with MeOH/CH_2Cl_2 mixtures) to afford the primary amine as a white solid (HPLC/MS-$[M+H]^+$ = 786.45).

The amine was dissolved in CH_2Cl_2 and treated with TFA. After stirring for 1 h, the reaction mixture was concentrated *in vacuo*. The off-white solid residue was dissolved in minimal amount of MeOH, cooled to 0 °C and treated with ether to precipitate out the desired diamine product as an off-white solid in 6% yield over four synthetic steps. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.50 (d, J = 8.6 Hz, 2H), 7.37 (s, 5H), 7.09 (d, J = 8.6 Hz, 2H), 6.41 (d, J = 9.4 Hz, 1H), 5.02 (t, J = 10.0 Hz, 1H), 4.91 (s, 1H), 4.70 (s, 2H), 4.27 (t, J = 5.0 Hz, 2H), 3.40 (t, J = 5.0 Hz, 2H), 3.37 (s, 1H), 3.12 (s, 3H), 2.47 (s, 3H), 2.06-1.95 (m, 1H), 1.94 (d, J = 1.4 Hz, 3H), 1.45 (s, 3H), 1.37 (s, 3H), 1.08 (s, 9H), 0.89 (dd, J = 9.7, 6.6 Hz, 6H). $C_{38}H_{55}N_5O_6S$ calcd. m/z = 685.39 amu; found $[M+H]^+$ = 686.32, $[M+Na]^+$ = 708.27, $[(M+2H)/2]^{2+}$ = 343.77.

Example 3.110: (*S*,*E*)-2,5-Dimethyl-*N*-(2-(2,2,2-trifluoroacetamido)phenylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2,2,2-trifluoro-*N*-(2-sulfamoylphenyl)acetamide according to General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.27 (d, J = 8.4 Hz, 1H), 8.05 (d, J = 7.8 Hz, 1H), 7.67 (t, J = 7.9 Hz, 1H), 7.54 (d, J = 8.1 Hz, 2H), 7.48 (t, J = 7.7 Hz, 2H), 7.40 (dt, J = 13.3, 7.4 Hz, 2H), 6.57 (d, J = 9.2 Hz, 1H), 4.92 (s, 2H), 4.34 (s, 1H), 3.17 (s, 3H), 2.50 (s, 3H), 2.06 (m, 1H), 1.87 (d, J = 1.3 Hz, 3H), 1.45 (s, 3H), 1.33 (s, 3H), 1.07 (s, 9H), 0.91 (dd, J = 6.6, 3.5 Hz, 6H). ^{19}F NMR (377 MHz, Methanol-d_4) δ-76.96,-77.73. $C_{35}H_{48}F_3N_5O_6S$ calcd. m/z = 723.33 amu; found $[M+H]^+$ = 723.34, [M+Na]+ = 746.23.

Example 3.111: (*S*,*E*)-*N*-(2-Aminophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2,2,2-trifluoro-*N*-(2-sulfamoylphenyl)acetamide according to General Procedures 9, 10 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.75 (dd, J = 8.2, 1.5 Hz, 1H), 7.55 (d, J = 7.8 Hz, 2H), 7.48 (t, J = 7.7 Hz, 2H), 7.38 (t, J = 7.4 Hz, 1H), 7.33-7.27 (m, 1H), 6.81 (d, J = 8.2 Hz, 1H), 6.69 (t, J = 7.5 Hz, 1H), 6.49 (dd, J = 9.1, 1.5 Hz, 1H), 4.97 (t, J = 10.1 Hz, 1H), 4.92 (s, 1H), 4.35 (s, 1H), 3.17 (s, 3H), 2.51 (s, 3H), 2.07 (m, 1H), 1.88 (d, J = 1.4 Hz, 3H), 1.46 (s, 3H), 1.36 (s, 3H), 1.06 (s, 9H), 0.92 (t, J = 6.8 Hz, 6H). $C_{33}H_{49}N_5O_5S$ calcd. m/z = 627.35 amu; found $[M+H]^+$ = 628.36, [M+Na]+ = 650.37, $[(M+2H)/2]^{2+}$ = 314.76.

Example 3.112: (*S*,*E*)-*N*-(Biphenyl-4-ylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared using from Boc protected Example 3.69 with phenylboronic acid according to General Procedures 13 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.12 (d, J = 8.3 Hz, 2H), 7.83 (d, J = 8.4 Hz, 2H), 7.71 (d, J = 7.7 Hz, 2H), 7.52 (dd, J = 11.6, 7.6 Hz, 4H), 7.45 (t, J = 7.3 Hz, 3H), 7.36 (t, J = 7.2 Hz, 1H), 6.52 (d, J = 9.4 Hz, 1H), 4.96 (t, J = 9.5 Hz, 1H), 4.92 (s, 1H), 4.33 (s, 1H), 3.18 (s, 3H), 2.50 (s, 3H), 2.14-2.03 (m, 1H), 1.88 (s, 3H), 1.45 (s, 3H), 1.35 (s, 3H), 1.07 (s, 9H), 0.92 (t, J = 6.9 Hz, 6H). $C_{39}H_{52}N_4O_5S$ calcd. m/z = 688.37 amu; found $[M+H]^+$ = 689.10, $[M+Na]^+$ = 711.32.

Example 3.113: (*S*,*E*)-*N*-(4'-Aminobiphenyl-4-ylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Boc protected Example 3.81 with 4-(*tert*-butoxycarbonylamino)phenylboronic acid according to General Procedures 13 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.05 (d, J = 8.6 Hz, 2H), 7.75 (d, J = 8.6 Hz, 2H), 7.59-7.51 (m, 4H), 7.45 (t, J

= 7.7 Hz, 2H), 7.36 (t, J = 7.3 Hz, 1H), 6.91 (d, J = 8.3 Hz, 2H), 6.50 (d, J = 9.1 Hz, 1H), 4.98-4.92 (m, 1H), 4.91 (s, 1H), 4.34 (s, 1H), 3.18 (s, 3H), 2.50 (s, 3H), 2.13-2.03 (m, 1H), 1.88 (d, J = 1.4 Hz, 3H), 1.45 (s, 3H), 1.35 (s, 3H), 1.06 (s, 9H), 0.92 (t, J = 6.2 Hz, 6H). $C_{39}H_{53}N_5O_5S$ calcd. m/z = 703.38 amu; found $[M+H]^+$ = 704.26, [M+Na]+ = 726.41, $[(M+2H)/2]^{2+}$ = 352.77.

Example 3.114: (*S*,*E*)-*N*-(4-Fluorobenzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 4-fluorobenzylsulfonamide according to General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.60-7.52 (m, 2H), 7.48 (t, J = 7.7 Hz, 2H), 7.44-7.34 (m, 3H), 7.18-7.05 (m, 2H), 6.41 (dd, J = 9.5, 1.7 Hz, 1H), 5.06 (t, J = 10.0 Hz, 1H), 4.94 (s, 1H), 4.74 (s, 2H), 4.35 (s, 1H), 3.13 (s, 3H), 2.51 (s, 3H), 2.07-1.97 (m, 1H), 1.95 (d, J = 1.4 Hz, 3H), 1.48 (s, 3H), 1.39 (s, 3H), 1.09 (s, 9H), 0.90 (t, J = 6.3 Hz, 6H). $C_{34}H_{49}FN_4O_5S$ calcd. m/z= 644.34 found $[M+H]^+$ = 645.32.

Example 3.115: (*S*,*E*)-2,5-Dimethyl-*N*-(3-(trifluoromethyl)benzylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 3-trifluorobenzylsulfonamide according to General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.74-7.64 (m, 3H), 7.61 (d, J = 7.7 Hz, 1H), 7.60-7.54 (m, 2H), 7.48 (t, J = 7.7 Hz, 2H), 7.38 (t, J = 7.3 Hz, 1H), 6.42 (dd, J = 9.4, 1.7 Hz, 1H), 5.06 (t, J = 10.0 Hz, 1H), 4.93 (s, 1H), 4.36 (s, 1H), 3.13 (s, 3H), 2.51 (s, 3H), 2.07-1.97 (m, 1H), 1.95 (d, J = 1.4 Hz, 3H), 1.48 (s, 3H), 1.39 (s, 3H), 1.08 (s, 9H), 0.89 (d, J = 6.5 Hz, 6H). $C_{35}H_{49}F_3N_4O_5S$ calcd. m/z = 694.34 found $[M+H]^+$ = 695.38.

Example 3.116: (*S*,*E*)-2,5-Dimethyl-*N*-(3-(trifluoromethoxy)benzylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 3-trifluoromethoxybenzylsulfonamide according to General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.56 (d, J = 7.8 Hz, 2H), 7.48 (t, J = 7.9 Hz, 3H), 7.43-7.36 (m, 2H), 7.32 (d, J = 9.3 Hz, 2H), 6.43 (dd, J = 9.4, 1.7 Hz, 1H), 5.06 (t, J = 10.0 Hz, 1H), 4.93 (s, 1H), 4.82 (s, 2H), 4.35 (s, 1H), 3.13 (s, 3H), 2.51 (s, 3H), 2.07-1.97 (m, 1H), 1.95 (d, J = 1.4 Hz, 3H), 1.48 (s, 3H), 1.39 (s, 3H), 1.08 (s, 9H), 0.90 (dd, J = 6.6, 4.3 Hz, 6H). $C_{35}H_{49}F_3N_4O_6S$ calcd. m/z = 710.33 found $[M+H]^+$ = 711.38.

Example 3.117: (*S*,*E*)-*N*-(3,4-Dichlorobenzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 3,4-dichlorobenzylsulfonamide according to General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.56 (td, J = 5.2, 4.5,

1.9 Hz, 4H), 7.48 (t, J = 7.7 Hz, 2H), 7.38 (t, J = 7.3 Hz, 1H), 7.33 (dd, J = 8.4, 2.1 Hz, 1H), 6.41 (dd, J = 9.5, 1.8 Hz, 1H), 5.06 (t, J = 10.0 Hz, 1H), 4.93 (s, 1H), 4.77 (s, 2H), 4.36 (s, 1H), 3.14 (s, 3H), 2.52 (s, 3H), 2.07-1.97 (m, 1H), 1.95 (d, J = 1.4 Hz, 3H), 1.49 (s, 3H), 1.39 (s, 3H), 1.08 (s, 9H), 0.90 (dd, J = 6.6, 4.9 Hz, 6H). $C_{34}H_{48}Cl_2N_4O_5S$ calcd. m/z = 694.27 found $[M+H]^+$ = 695.32.

Example 3.118: (*S*,*E*)-*N*-(2-Cyanobenzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2-cyanobenzylsulfonamide according to General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.81 (dd, J = 7.7, 1.3 Hz, 1H), 7.72 (td, J = 7.7, 1.3 Hz, 1H), 7.66 (d, J = 7.7 Hz, 1H), 7.62-7.59 (m, 1H), 7.58-7.53 (m, 2H), 7.48 (t, J = 7.7 Hz, 2H), 7.38 (t, J = 7.3 Hz, 1H), 6.50 (d, J = 9.4 Hz, 1H), 5.08 (dd, J = 10.6, 9.3 Hz, 1H), 4.99 (s, 2H), 4.95 (s, 1H), 4.36 (s, 1H), 3.16 (s, 3H), 2.52 (s, 3H), 2.09-1.99 (m, 1H), 1.98 (d, J = 1.4 Hz, 3H), 1.49 (s, 3H), 1.39 (s, 3H), 1.10 (s, 9H), 0.94 (d, J = 6.6 Hz, 3H), 0.91 (d, J = 6.6 Hz, 3H). $C_{35}H_{49}N_5O_5S$ calcd. m/z = 651.35 found $[M+H]^+$ = 652.38.

Example 3.119: (*S*,*E*)-*N*-(3-Chlorobenzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 3-chlorobenzylsulfonamide according to General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.58-7.53 (m, 2H), 7.48 (t, J = 7.6 Hz, 2H), 7.43-7.34 (m, 4H), 7.32 (d, J = 7.5 Hz, 1H), 6.42 (d, J = 9.5 Hz, 1H), 5.06 (t, J = 10.0 Hz, 1H), 4.94 (s, 1H), 4.74 (s, 2H), 4.33 (s, 1H), 3.13 (s, 3H), 2.50 (s, 3H), 2.07-1.97 (m, 1H), 1.95 (d, J = 1.4 Hz, 3H), 1.48 (s, 3H), 1.39 (s, 3H), 1.08 (s, 9H), 0.90 (t, J = 7.2 Hz, 6H). $C_{34}H_{49}ClN_4O_5S$ calcd. m/z = 660.31 found $[M+H]^+$ = 661.32.

Example 3.120: (107) (*S*,*E*)-*N*-(4-Amino-2-ethylphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2-ethylbenzylsulfonamide according to General Procedures 9 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.79 (d, J = 8.7 Hz, 1H), 7.55 (d, J = 7.9 Hz, 2H), 7.48 (t, J = 7.6 Hz, 2H), 7.37 (t, J = 7.4 Hz, 1H), 6.57 (d, J = 2.3 Hz, 1H), 6.54 (dd, J = 8.8, 2.4 Hz, 1H), 6.46 (d, J = 9.4 Hz, 1H), 5.01 (t, J = 10.0 Hz, 1H), 4.92 (s, 1H), 4.34 (s, 1H), 3.16 (s, 3H), 2.99-2.90 (m, 2H), 2.50 (s, 3H), 2.11-2.00 (m, 1H), 1.87 (d, J = 1.4 Hz, 3H), 1.47 (s, 3H), 1.38 (s, 3H), 1.22 (t, J = 7.5 Hz, 3H), 1.06 (s, 9H), 0.91 (dd, J = 6.6 Hz, 6H). $C_{35}H_{53}N_5O_5S$ calcd. m/z = 655.38 found $[M+H]^+$ = 656.4.

Example 3.121: (*S*,*E*)-*N*-(4-Amino-3-(trifluoromethoxy)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2,2,2-trifluoro-*N*-(4-sulfamoyl-2-(trifluoromethoxy)phenyl)acetamide according to General Procedures 9, 10 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.81-7.75 (m, 1H), 7.71 (dd, J = 8.7, 2.1 Hz, 1H), 7.55 (d, J = 7.9 Hz, 2H), 7.47 (t, J = 7.6 Hz, 2H), 7.37 (t, J = 7.1 Hz, 1H), 6.89 (d, J = 8.7 Hz, 1H), 6.51-6.42 (m, 1H), 4.98 (t, J = 10.0 Hz, 1H), 4.92 (t, J = 4.1 Hz, 1H), 4.37 (s, 1H), 3.16 (s, 3H), 2.51 (s, 3H), 2.12-2.01 (m, 1H), 1.88 (d, J = 1.4 Hz, 3H), 1.47 (s, 3H), 1.37 (s, 3H), 1.07 (s, 9H), 0.92 (dd, J = 6.6 Hz, 6H). $C_{34}H_{48}F_3N_5O_6S$ calcd. m/z = 711.33 found $[M+H]^+$ = 712.4.

Example 3.122: (*S*,*E*)-*N*-(4-Amino-2,3-dimethylphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2,2,2-trifluoro-*N*-(4-sulfamoyl-2,3-dimethylphenyl)acetamide according to General Procedures 9, 10 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.75 (d, J = 8.8 Hz, 1H), 7.55 (d, J = 7.9 Hz, 2H), 7.47 (t, J = 7.7 Hz, 2H), 7.37 (t, J = 6.9 Hz, 1H), 6.63 (d, J = 8.8 Hz, 1H), 6.46 (d, J = 9.7 Hz, 1H), 5.00 (t, J = 10.0 Hz, 1H), 4.93 (s, 1H), 4.32 (s, 1H), 3.17 (s, 3H), 2.54 (s, 3H), 2.49 (s, 3H), 2.09 (s, 3H), 2.08-2.02 (m, 1H), 1.87 (d, J = 1.4 Hz, 3H), 1.47 (s, 3H), 1.37 (s, 3H), 1.07 (s, 9H), 0.92 (dd, J = 6.8, 6.5 Hz, 6H). $C_{35}H_{53}N_5O_5S$ calcd. m/z = 655.38 found $[M+H]^+$ = 656.4.

Example 3.123: (*S*,*E*)-*N*-(4-Amino-5,6,7,8-tetrahydronaphthalen-1-ylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2,2,2-trifluoro-*N*-(4-sulfamoyl-5,6,7,8-tetrahydronaphthalen-1-yl)acetamide according to General Procedures 9, 10 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.74 (d, J = 8.7 Hz, 1H), 7.55 (d, J = 7.9 Hz, 2H), 7.48 (t, J = 7.6 Hz, 2H), 7.38 (t, J = 7.2 Hz, 1H), 6.60 (d, J = 8.7 Hz, 1H), 6.46 (d, J = 9.2 Hz, 1H), 5.00 (t, J = 10.0 Hz, 1H), 4.95-4.91 (m, 1H), 4.36 (s, 1H), 3.17 (s, 3H), 3.10-3.05 (m, 2H), 2.51 (s, 3H), 2.46 (t, J = 6.5 Hz, 2H), 2.10-2.02 (m, 1H), 1.88 (s, 3H), 1.87-1.75 (m, 4H), 1.47 (s, 3H), 1.38 (s, 3H), 1.07 (s, 9H), 0.92 (dd, J = 7.1 Hz, 6H). $C_{37}H_{55}N_5O_5S$ calcd. m/z = 681.39 found $[M+H]^+$ = 682.4.

Example 3.124: (*S*,*E*)-*N*-(4-Amino-3-methylphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2,2,2-trifluoro-*N*-(2-methyl-4-sulfamoylphenyl)acetamide according to General Procedures 9, 10 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.64 (s, 1H), 7.61 (dd, J = 8.5, 2.3 Hz, 1H), 7.57-7.51 (m, 2H), 7.48 (t, J = 7.7 Hz, 2H), 7.41-7.35 (m, 1H), 6.71 (d, J = 8.5 Hz, 1H), 6.43 (dd, J = 9.3, 1.6 Hz, 1H), 4.96 (t, J = 10.0 Hz, 1H), 4.92 (s, 1H), 4.35 (s, 1H), 3.16 (s, 3H), 2.51 (s, 3H), 2.17 (s, 3H), 2.10-2.01 (m, 1H), 1.87 (d, J =

1.4 Hz, 3H), 1.46 (s, 3H), 1.36 (s, 3H), 1.07 (s, 9H), 0.91 (dd, J = 6.3 Hz, 6H). $C_{34}H_{51}N_5O_5S$ calcd. m/z = 641.36 found $[M+H]^+$ = 642.4.

Example 3.125: (*S*,*E*)-*N*-(4-Amino-3-fluorophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2,2,2-trifluoro-*N*-(2-fluoro-4-sulfamoylphenyl)acetamide according to General Procedures 9, 10 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.62-7.55 (m, 3H), 7.54 (s, 1H), 7.48 (t, J = 7.7 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 6.85 (t, J = 8.6 Hz, 1H), 6.45 (d, J = 9.3 Hz, 1H), 4.98 (t, J = 9.9 Hz, 1H), 4.92 (s, 1H), 4.34 (s, 1H), 3.16 (s, 3H), 2.50 (s, 3H), 2.12-2.00 (m, 1H), 1.88 (d, J = 1.4 Hz, 3H), 1.46 (s, 3H), 1.37 (s, 3H), 1.07 (s, 9H), 0.91 (dd, J = 6.8 Hz, 6H). $C_{33}H_{48}FN_5O_5S$ calcd. m/z = 645.34 found $[M+H]^+$ = 646.4.

Example 3.126: (*S*,*E*)-*N*-(4-Amino-3-ethylphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2,2,2-trifluoro-*N*-(2-ethyl-4-sulfamoylphenyl)acetamide according to General Procedures 9, 10 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.66 (d, J = 2.3 Hz, 1H), 7.61 (dd, J = 8.6, 2.3 Hz, 1H), 7.55 (d, J = 7.6 Hz, 2H), 7.48 (t, J = 7.7 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 6.71 (d, J = 8.5 Hz, 1H), 6.43 (dd, J = 9.3, 1.7 Hz, 1H), 4.96 (t, J = 9.9 Hz, 1H), 4.92 (s, 1H), 4.35 (s, 1H), 3.16 (s, 3H), 2.54 (dd, J = 7.4, 2.2 Hz, 2H), 2.51 (s, 3H), 2.12-1.99 (m, 1H), 1.87 (d, J = 1.4 Hz, 3H), 1.46 (s, 3H), 1.36 (s, 3H), 1.27 (t, J = 7.5 Hz, 3H), 1.07 (s, 9H), 0.91 (dd, J = 6.4 Hz, 6H) $C_{35}H_{53}N_5O_5S$ calcd. m/z = 655.38 found $[M+H]^+$ = 656.5.

Example 3.127: (*S*,*E*)-*N*-(4-Amino-3-(trifluoromethyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

Title compound was prepared from Example 3.16 and 2,2,2-trifluoro-*N*-(2-trifluoromethyl-4-sulfamoylphenyl)acetamide according to General Procedures 9, 10 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.04 (s, 1H), 7.87 (d, J = 8.8 Hz, 1H), 7.55 (d, J = 7.6 Hz, 2H), 7.48 (t, J = 7.3 Hz, 2H), 7.36 (dd, J = 14.5, 7.4 Hz, 1H), 6.89 (d, J = 8.9 Hz, 1H), 6.47 (d, J = 9.3 Hz, 1H), 4.99 (t, J = 10.2 Hz, 1H), 4.92 (s, 1H), 4.33 (s, 1H), 3.16 (s, 3H), 2.50 (s, 3H), 2.11-2.00 (m, 1H), 1.88 (s, 3H), 1.47 (s, 3H), 1.37 (s, 3H), 1.07 (s, 9H), 0.91 (dd, J = 7.0 Hz, 6H). $C_{34}H_{48}F_3N_5O_5S$ calcd. m/z = 695.33 found $[M+H]^+$ = 696.4.

Example 3.128: (*S*)-1-Isopropyl-*N*-((*S*)-1-(((*S*,*E*)-6-(3-mercaptopropylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-2-carboxamide.

To a solution of (*S*,*E*)-ethyl 4-((*S*)-2-(*tert*-butoxycarbonylamino)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enoate (0.373g, 0.905mmol) in CH_2Cl_2 (5mL) was added trifluoroacetic acid (2 mL). The reaction was monitored by HPLC and upon complete conversion of the starting material concentrated under reduced pressure. *N*-isopropyl-pipecolic acid (0.200g, 1.3 equiv) was dissolved in CH_2Cl_2 (5mL) and stirred at 0 °C, to which was added HBTU (0.450g, 1.3 equiv) and *N*,*N*-di-isopropylethylamine (0.400 µL, 2.5 equiv). After 10 minutes, the above deprotected dipeptide was added as a solution in CH_2Cl_2 (~1mL). The reaction was monitored by HPLC for complete consumption of the dipeptide at which time the entire reaction was concentrated under reduced pressure. The crude reaction mixture was dissolved in CH_2Cl_2 and purified by silica gel chromatography (1-20% MeOH (5% NH_4OH) in CH_2Cl_2).

The resulting ester was saponified with LiOH in 1,4-dioxane. The resulting carboxylic acid (0.128g, 0.29mmol) was dissolved in CH_2Cl_2 (5mL) and to the stirred solution was added dicyclohexylcarbodiimide (0.084g, 1.4 equiv), *N*,*N*-dimethylaminopyridine (0.05g, 1.4 equiv) and 3-(tritylthio)propane-1-sulfonamide (0.174g, 1.5 equiv). The resulting mixture was stirred overnight and monitored for reaction progress by HPLC-MS. When the reaction was complete, the mixture was concentrated under reduced pressure and the residue was purified by silica gel chromatography (5-30% MeOH in CH_2Cl_2) to give the *S*-trityl derivative of the parent compound as a colorless oil (0.056g).^{1}H NMR (400 MHz, Methanol-d_4) δ 7.44-7.35 (m, 6H), 7.36-7.15 (m, 9H), 6.56 (dd, *J* = 9.1, 1.7 Hz, 1H), 5.03 (dd, *J* = 10.6, 9.3 Hz, 1H), 4.73 (s, 1H), 4.05 (dd, *J* = 11.5, 3.3 Hz, 1H), 3.51-3.37 (m, 2H), 3.25-3.15 (m, 2H), 3.09 (s, 3H), 2.92 (td, *J* = 12.5, 2.9 Hz, 1H), 2.31 (t, *J* = 7.2 Hz, 2H), 2.18-1.70 (m, 15H), 1.61 (ddt, *J* = 12.8, 8.4, 4.9 Hz, 1H), 1.28 (dd, *J* = 30.1, 6.7 Hz, 7H), 1.04 (s, 9H), 0.88 (dd, *J* = 37.3, 6.5 Hz, 6H).

Finally, the trityl protected thiol was dissolved in CH_2Cl_2 (3 mL) and trifluoroacetic acid was added (0.6 mL) with triisopropyl silane (0.1mL). The reaction was monitored by HPLC-MS and upon completion, was concentrated to dryness under reduced pressure. The residue was taken up in CH_2Cl_2 (~0.8mL) with a couple of drops of ethanol and cooled to 0 °C in an ice bath. Cold diethyl ether (~3mL) was added with vigorous stirring to generate a white precipitate which was collected by filtration on a Buchner funnel at dried under high vacuum to yield the parent compound as an amorphous white solid. ^{1}H NMR (400 MHz, Methanol-d_4) δ 6.52 (d, *J* = 9.0 Hz, 1H), 5.06 (dd, *J* = 10.7, 8.8 Hz, 1H), 4.73 (s, 1H), 4.16-4.04 (m, 1H), 3.69-3.56 (m, 2H), 3.48 (dd, *J* = 13.3, 7.2 Hz, 2H), 3.15 (s, 3H), 3.03-2.94 (m, 1H), 2.68 (t, *J* = 6.9 Hz, 1H), 2.24-1.77 (m, 11H), 1.61 (s, 1H), 1.31 (dd, *J* = 27.2, 6.7 Hz, 6H), 1.06 (s, 9H), 0.91 (dd, *J* = 34.1, 6.6 Hz, 6H).

Example 3.129: (S)-*N*-((*S*)-1-((*S*)-2-((*E*)-3-(3-Mercaptopropylsulfonamido)-2-methyl-3-oxoprop-1-enyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-3-methyl-2-(methylamino)-3-phenylbutanamide.

The title compound was synthesized from Boc-proline and Example 3.15 according to General Procedures 15, 16, 9, 10, 12 and others from Nieman J. A. *et al.* J. Nat. Prod. 2003, 66, 183-199. The compound was isolated as two diastereoisomers in an approximately 1:1 ratio. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.57-7.12 (m, 5H), 6.39 (dd, J = 9.4, 1.6 Hz, 0.5H), 6.31 (dd, J = 8.2, 1.5 Hz, 0.5H), 4.72 (q, J = 7.5 Hz, 0.5H), 4.66-4.56 (m, 0.5H), 4.40 (s, 0.5H), 4.28 (d, J = 11.9 Hz, 1H), 3.81 (m, 0.5H), 3.76-3.56 (m, 3H), 2.77-2.64 (m, 2H), 2.59 (m, 3H), 2.39-2.22 (m, 1H), 2.18-1.72 (m, 7H), 1.61-1.33 (m, 6H), 1.15-0.85 (m, 11H). $C_{29}H_{46}N_4O_5S_2$ calcd. m/z = 594.35 found $[M+H]^+$ = 595.3.

Example 3.130: (*S*)-*N*-((*S*)-1-(2-(3-(3-Mercaptopropylsulfonamido)-2-methyl-3-oxoprop-1-enyl)piperidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-3-methyl-2-(methylamino)-3-phenylbutanamide.

The title compound was synthesized from Boc-homoproline and Example 3.15 according to General Procedures 15, 16, 9, 10, 12 and others from Nieman J. A. *et al.* J. Nat. Prod. 2003, 66, 183-199. The compound was isolated as two diastereoisomers in an approximately 2:3 ratio. ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.55 (d, J = 7.8 Hz, 1H), 7.46 (m, 3H), 7.38 (m, 1H), 6.81 (d, J = 8.3 Hz, 0.6H), 6.79 (d, J = 7.8 Hz, 0.4H), 5.66 (m, 0.6H), 5.12 (m, 0.4H), 5.05 (s, 0.6H), 4.86 (s, 0.4H), 4.42 (d, J = 14.9 Hz, 0.4H), 4.35 (s, 0.6H), 4.26 (s, 0.4H), 4.12 (d, J = 13.8 Hz, 0.6H), 3.64 (d, J = 7.6 Hz, 1H), 3.63 (d, J = 7.4 Hz, 1H), 3.39 (m, 0.6H), 2.94 (td, J = 13.8, 2.6 Hz, 0.4H), 2.68 (t, J = 6.7 Hz, 2H), 2.56 (m, 3H), 2.10 (m, 3.5H), 1.97 (s, 1.5H), 1.90–1.70 (m, 7H), 1.65–1.29 (m, 6H), 1.07 (s, 3.5H), 1.04 (s, 4.5H) ppm. $C_{30}H_{47}N_4O_5S_2$ calcd. m/z = 608.31; found $[M+H]^+$ = 609.32.

Example 3.131: (*S*)-*N*-((*S*)-1-(2-(3-(4-(Mercaptomethyl)phenylsulfonamido)-2-methyl-3-oxoprop-1-enyl)piperidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-3-methyl-2-(methylamino)-3-phenylbutanamide.

The title compound was synthesized from Boc-homoproline and Example 3.20 according to General Procedures 15, 16, 9, 10, 12 and others from Nieman J. A. *et al.* J. Nat. Prod. 2003, 66, 183-199. The compound was isolated as two diastereoisomers in an approximately 2:3 ratio. ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.02 (d, J = 8.4 Hz, 0.8H), 8.00 (d, J = 8.5 Hz, 1.2H), 7.58 (d, J = 8.5 Hz, 1H), 7.54 (d, J = 8.5 Hz, 2H), 7.45 (t, J = 8.2 Hz, 2H), 7.40 (d, J = 7.2 Hz, 0.6H), 7.36 (m, 1H), 7.31 (t, J = 7.1 Hz, 0.4H), 6.74 (d, J = 8.2 Hz, 1H), 5.59 (m, 0.6H), 5.06 (m, 0.4H), 5.02 (s, 0.6H), 4.84 (s, 0.4H), 4.39 (d, J = 12.5 Hz, 0.4H), 4.34 (s, 0.6H), 4.20 (s, 0.4H), 4.08 (d, J = 12.0 Hz, 0.6H), 3.83 (s, 1.2H), 3.73 (s, 0.8H), 3.35 (m, 0.6H), 2.93 (td, J = 13.6, 3.0 Hz, 0.4H), 2.55 (m, 3H), 2.00 (s, 1H), 1.90–1.51 (m, 7H), 1.51–1.30 (m, 4H), 1.30 (s, 1H), 1.15 (s, 1H), 1.04 (s, 3.5H), 1.01 (s, 4.5H) ppm. $C_{34}H_{47}N_4O_5S_2$ calcd. m/z = 656.31; found $[M+H]^+$ = 657.30.

Example 3.132: MC-VC-PABC-3.90.

The title compound was prepared by application of General Procedures 20 and 12 from Boc protected Example 3.90. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.58 (d, J = 8.2 Hz, 2H), 7.49 (d, J = 7.5 Hz, 2H), 7.38 (t, J = 7.7 Hz, 2H), 7.36-7.24 (m, 6H), 7.22 (d, J = 7.8 Hz, 2H), 6.81 (s, 2H), 6.57 (d, J = 9.1 Hz, 1H), 5.08 (s, 2H), 5.04 (t, J = 10.0 Hz, 1H), 4.91 (s, 1H), 4.53 (dd, J = 9.0, 5.1 Hz, 1H), 4.40 (s, 2H), 4.28 (s, 2H), 4.19 (d, J = 7.4 Hz, 1H), 3.49 (t, J = 7.1 Hz, 2H), 3.26-3.11 (m, 2H), 3.07-2.93 (m, 3H), 2.30 (t, J = 7.4 Hz, 2H), 2.18 (s, 3H), 2.15-2.05 (m, 1H), 1.99-1.91 (m, 1H), 1.89 (s, 3H), 1.83-1.72 (m, 1H), 1.72-1.53 (m, 7H), 1.44 (s, 3H), 1.37 (s, 3H), 1.35-1.27 (m, 2H), 1.03 (s, 9H), 1.00 (d, J = 6.8 Hz, 3H), 0.99 (d, J = 6.7 Hz, 3H), 0.88 (d, J = 6.5 Hz, 3H), 0.82 (d, J = 6.6 Hz, 3H). $C_{64}H_{91}N_{11}O_{13}S$ calcd. m/z = 1253.7; found $[M+H]^+$ = 1254.8.

Example 3.133: 4-((*R*)-2-((*R*)-2-(6-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-(*N*-((*S*,*E*)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enoyl)sulfamoyl)benzylcarbamate (MC-VC-PABC-3.98).

The title compound was prepared by application of General Procedures 20 and 12 to Boc protected Example 3.98. $C_{63}H_{89}N_{11}O_{13}S$ calcd. m/z = 1239.6; found $[M+H]^+$ = 1240.9.

Example 3.134: MC-VC-PABC-3.93.

The title compound was prepared by application of General Procedures 20 and 12 to Boc protected Example 3.93. $C_{63}H_{89}N_{11}O_{13}S$ calcd. m/z = 1239.6; found $[M+H]^+$ = 1240.9.

Example 3.135: MC-VC-PABC-3.54.

The title compound was prepared by application of General Procedure 20 to Example 3.54. $C_{64}H_{91}N_{11}O_{13}S$ calcd. m/z = 1253.65; found $[M+H]^+$ = 1254.75, $[M+2H]_{2+}$ = 628.20.

Example 3.136: (*R*)-*N*-(Benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hexanamide.

A suspension of the Example 3.27 and 10% palladium on carbon (25 mol% Pd) in glacial acetic acid was stirred under a H_2 atmosphere (1 atm) at ambient temperature. After 142 h, the reaction suspension was passed through a bed of Celite, rinsed with MeOH (5×) and concentrated *in vacuo*. The residual light brown crude film was dissolved and purified on the preparative HPLC (30-70% MeCN/H_2O with 0.1% TFA) and lyophilized to afford one diastereomer of the reduced product as a pale yellow solid in 15% yield ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.55 (d, J = 7.2 Hz, 2H), 7.46 (t, J = 7.8 Hz, 2H), 7.43-7.31 (m, 6H), 5.01 (s, 1H), 4.79 (d, J = 14.1 Hz, 1H), 4.65 (d, J = 14.1 Hz, 1H), 4.35 (s, 1H), 4.24 (s, 1H), 3.07 (s, 3H), 2.52 (s, 3H), 2.27 (m, J = 10.3, 7.0, 3.2 Hz, 1H), 2.14 (ddd, J = 13.5, 10.6, 2.7 Hz, 2H), 1.78 (d, J = 8.6 Hz, 1H), 1.47 (s, 3H), 1.34 (s, 3H), 1.15 (d, J = 6.9

Hz, 3H), 1.14 (s, 9H), 1.04 (d, J = 6.6 Hz, 3H), 0.82 (d, J = 6.6 Hz, 3H). $C_{34}H_{52}N_4O_5S$ calcd. m/z = 628.37 amu; found $[M+H]^+$ = 629.6, $[M+Na]^+$ = 651.

Example 3.137: 3-Methyl-3-(4-bromophenyl)-butanoic Acid.

To a vigorously stirred solution of bromobenzene (4.70 g, 30.0 mmol) and 3,3-dimethylacrylic acid (1.00 g, 10.0 mmol) in 20 mL CH_2Cl_2 cooled to -10 °C in an NH_4Cl(aq)/ice bath, solid $AlCl_3$ was added portion-wise, keeping the internal temperature below -5 °C. The solution turned yellow, then brown after addition. After one hour, analysis by LC and TLC indicated complete consumption of the limiting reagent. The reaction was then quenched by the addition of 1 M citric acid, causing the brown color to fade to yellow. The resulting sloppy suspension was extracted four times with 20 mL Et_2O, the combined organics washed with NaCl(sat), dried over Na_2SO_4(s), and concentrated *in vacuo* with heating to 45 °C to remove solvent and residual bromobenzene. The resulting oil solidified slowly. Recrystallization of the crude solid in hexanes afforded the title compound (1.29 g, 50%) as clusters of white prisms. ^{1}H NMR (400 MHz, Chloroform-*d*) δ (ppm) 7.42 (d, J = 8.6 Hz, 2H), 7.23 (d, J = 8.6 Hz, 2H), 2.63 (s, 2H), 1.43 (s, 6H). $C_{11}H_{13}BrO_2$ calcd. $[M+H]^+$ = 257.02 amu; found m/z = 257.03. R_f = 0.21 (20% (2% AcOH/EtOAc)/Hex).

Example 3.138: 3-Methyl-3-(3-bromophenyl)-butanoic acid.

The title compound was prepared in the same manner as 3-methyl-3-phenylbutanoic acid in Nieman J. A., *et al.* J. Nat. Prod. 2003, 66, 183-199, using bromobenzene in place of benzene as the solvent, and substituting the acid-base workup with a simple extraction of the reaction mixture from 1 M citric acid and three successive recrystallizations from hexanes. From a crude product enriched in the desired meta isomer as a 2:1 mixture, the title compound could be obtained as white stubby needles in greater than 95% purity. ^{1}H NMR (400 MHz, Chloroform-*d*) δ (ppm) 7.49 (t, J = 1.9 Hz, 1H), 7.34 (ddd, J = 7.9, 1.9, 1.0 Hz, 1H), 7.29 (ddd, J = 7.9, 1.9, 1.0 Hz, 1H), 7.18 (t, J = 7.9 Hz, 1H), 2.64 (s, 2H), 1.44 (s, 6H). $C_{11}H_{13}BrO_2$ calcd. $[M+H]^+$ = 257.02 amu; found m/z = 257.01. R_f = 0.21 (20% (2% AcOH/EtOAc)/Hex).

Example 3.139: (*S*)-Methyl 3-(4-bromophenyl)-2-(*tert*-butoxycarbonyl(methyl)amino)-3-methylbutanoate.

The title compound was synthesized from Example 3.137 according to the sequence of procedures described by Nieman *et al.* for the synthesis of (*S*)-methyl 2-(*tert*-butoxycarbonyl(methyl)amino)-3-methyl-3-phenylbutanoate.

Example 3.140: (*S*)-2-((*tert*-Butoxycarbonyl)(methyl)amino)-3-(4-((14-hydroxy-3,6,9,12-tetraoxatetradecyl)oxy)phenyl)-3-methylbutanoic acid.

To a stirred solution of Example 3.81 (157 mg, 0.405 mmol) in pentaethylene glycol (1.5 mL) were added $CsCO_3$ (330 mg, 1.01 mmol), 3,4,7,8-tetramethyl-1,10-phenanthroline (57 mg, 0.24 mmol), and CuI (23 mg, 0.12 mmol). Nitrogen was blown into the flask, then it was sealed and heated to 130 °C, the solution quickly turning red to brown to black. After 40 h, the reaction looked to be nearly complete by HPLC analysis. Thus, the mixture was allowed to cool to ambient temperature, diluted with H_2O, and transferred to a larger Erlenmeyer with a stir bar. This mixture was carefully acidified to pH ~ 3 with 1 M citric acid, paying attention not to allow the foamy mixture to spill over. The mixture was then extracted five times with CH_2Cl_2, the combined organic extracts washed with NaCl(sat), dried over Na_2SO_4(s), and concentrated *in vacuo* to yield about 300 mg of crude oil. Purification by flash chromatography (1-10% MeOH/(2% AcOH/EtOAc)) yielded the title compound (66 mg, 30%) as a clear film which existed as a set of *N*-Boc rotamers an approximate 2:1 ratio. ^{1}H NMR (400 MHz, Chloroform-*d*) δ (ppm) 7.35 (d, J = 7.8 Hz, 1.3H), 7.30 (d, J = 7.6 Hz, 0.7H), 6.87 (d, J = 7.1 Hz, 2H), 5.07 (s, 0.7H), 4.93 (s, 0.3H), 4.14 (m, 2H), 3.86 (m, 2H), 3.70 (m, 16H), 2.83 (s, 1H), 2.72 (s, 2H), 1.54 (s, 3H), 1.49 (s, 3H), 1.45 (s, 9H). $C_{27}H_{45}NO_{10}$ calcd. $[M+H]^+$ = 544.31 amu; found *m/z* = 544.36. R_f = 0.36 (5% MeOH/(2% AcOH/EtOAc)).

Example 3.141: (*S*)-2-((*tert*-Butoxycarbonyl)(methyl)amino)-3-(4-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethoxy)phenyl)-3-methylbutanoic acid.

The title compound was prepared according to the above method from Example 3.81 (132 mg, 0.341 mmol), $CsCO_3$ (278 mg, 0.853 mmol), 3,4,7,8-tetramethyl-1,10-phenanthroline (24 mg, 0.10 mmol), and CuI (10 mg, 0.051 mmol). Flash chromatography (1-10% MeOH/(2% AcOH/EtOAc)) gave the title compound (66 mg, 38%) as a clear oil in an approximate 2:1 ratio of *N*-Boc rotamers. ^{1}H NMR (400 MHz, Chloroform-*d*) δ (ppm) 7.34 (d, J = 8.4 Hz, 1.3H), 7.29 (d, J = 8.1 Hz, 0.7H), 6.85 (d, J = 8.4 Hz, 2H), 5.05 (s, 0.7H), 4.91 (s, 0.3H), 4.13 (t, J = 4.6 Hz, 2H), 3.87-3.79 (m, 2H), 3.76-3.60 (m, 10H), 3.59 (t, J = 4.1 Hz, 2H), 2.80 (s, 1H), 2.69 (s, 2H), 1.53 (s, 3H), 1.48 (s, 3H), 1.44 (s, 9H). $C_{25}H_{41}NO_9$ calcd. $[M+H]^+$ = 500.29 amu; found *m/z* = 500.36. R_f = 0.46 (5% MeOH/(2% AcOH/EtOAc)).

Example 3.142: (*S*)-3-(3-((14-Hydroxy-3,6,9,12-tetraoxatetradecyl)oxy)phenyl)-3-methyl-2-(methylamino)butanoic acid.

The precursor to the title compound, (*S*)-3-(3-bromophenyl)-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-methylbutanoic acid, was prepared from Example 3.138 by following the procedures in Neiman *et al.* Thus, following the procedures above, from (*S*)-3-(3-

bromophenyl)-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-methylbutanoic acid (166 mg, 0.43 mmol), $CsCO_3$ (330 mg, 1.01 mmol), 3,4,7,8-tetramethyl-1,10-phenanthroline (31 mg, 0.13 mmol), and CuI (12.3, 0.060 mmol) in 1.5 mL pentaethylene glycol heated to 130 °C for two days, the title compound (73 mg, 31%) was obtained as a clear oil after flash chromatography (1-10% MeOH/(2% AcOH/EtOAc)) in an approximate 2:1 ratio of *N*-Boc rotamers. ^{1}H NMR (400 MHz, Chloroform-*d*) δ (ppm) 7.17 (t, *J* = 7.8 Hz, 1H), 7.14-7.07 (m, 1H), 7.07-6.93 (m, 2H), 6.74 (d, *J* = 8.0 Hz, 1H), 5.11 (s, 0.7H), 4.93 (s, 0.3H), 4.25-4.03 (m, 2H), 3.91-3.77 (m, 2H), 3.78-3.66 (m, 2H), 3.69-3.43 (s, 14H), 2.72 (s, 1H), 2.65 (s, 1H), 1.51 (s, 3H), 1.49 (s, 3H), 1.45 (s, 9H). $C_{27}H_{45}NO_{10}$ calcd. $[M+H]^+$ = 544.31 amu; found *m/z* = 544.34.

Example 3.143: (6*S*,9*S*,12*S*,*E*)-Ethyl 9-(*tert*-butyl)-12-isopropyl-2,2,5,11,14-pentamethyl-4,7,10-trioxo-6-(2-(4-((16-oxo-3,6,9,12-tetraoxa-15-thiaheptadecyl)oxy)phenyl)propan-2-yl)-3-oxa-5,8,11-triazapentadec-13-en-15-oate.

(*S*)-2-((*tert*-Butoxycarbonyl)(methyl)amino)-3-(4-((14-hydroxy-3,6,9,12-tetraoxatetradecyl)oxy)phenyl)-3-methylbutanoic acid (65 mg, 0.120 mmol) was coupled to (*S*,*E*)-ethyl 4-((*S*)-2-amino-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enoate with HATU and DIPEA following the same stoichiometry and procedure as described in the general coupling procedures in Nieman *et al.* to give an intermediate free alcohol after purification by flash chromatography (1-10% MeOH/(2% AcOH/EtOAc)). Next, to triphenylphosphine (40 mg, 0.15 mmol) in 0.75 mL THF under N2 at 0 °C, di-*tert*-butylazodicarboxylate (35 mg, 0.15 mmol) was added in one portion. After 35 minutes, a white precipitate crashed out and the reaction became difficult to stir. To this suspension, a solution of the intermediate alcohol (42 mg, 0.050 mmol) in 0.75 mL THF was added diluting the precipitate enough to restore stirring. Five minutes later, thioacetic acid (5.7 mg, 0.075 mmol) in 0.05 mL THF was added causing all yellow color to fade from the mixture. After 30 min, the reaction was allowed to warm to ambient temperature. The precipitate disappeared after another 15 min, and analysis by TLC and LCMS showed nearly complete conversion. After another 40 minutes, the reaction mixture was concentrated *in vacuo*, then subjected directly to flash chromatography (40-100% EtOAc/Hex then to 10% MeOH/EtOAc) to yield the title compound (26 mg, 57%) as a clear film. ^{1}H NMR (400 MHz, Chloroform-*d*) δ (ppm) 7.43 (d, *J* = 8.4 Hz, 1.3H), 7.31 (d, *J* = 8.3 Hz, 0.7H), 6.97-6.72 (m, 2H), 6.62 (dd, *J* = 9.3, 1.6 Hz, 1H), 6.14 (d, *J* = 9.6 Hz, 1H), 5.22 (s, 0.7H), 5.12-4.99 (m, 1H), 4.84 (s, 0.3H), 4.69 (d, *J* = 9.3 Hz, 0.3H), 4.60 (d, *J* = 8.9 Hz, 0.7H), 4.19 (q, *J* = 7.2 Hz, 2H), 4.09 (td, *J* = 4.6, 2.3 Hz, 2H), 3.84 (t, *J* = 4.9 Hz, 2H), 3.77-3.70 (m, 2H), 3.70-3.61 (m, 10H), 3.59 (t, *J* = 6.4 Hz, 2H), 3.07 (t, *J* = 6.4 Hz, 2H), 2.97-2.91 (m, 3H), 2.84 (s, 3H), 2.32 (s, 3H), 1.87 (s, 3H), 1.49 (s, 3H), 1.43 (s, 9H), 1.35 (s, 3H), 1.30 (t, *J* = 7.1 Hz, 3H), 0.87 (d, *J* = 6.6 Hz, 3H), 0.80 (d, *J* = 16.6 Hz, 3H), 0.77 (s, 9H). $C_{46}H_{77}N_3O_{12}S$ calcd. $[M+H]^+$ = 896.53 amu; found *m/z* = 896.77. R_f = 0.56 (80% EtOAc/Hex).

Example 3.144: (6*S*,9*S*,12*S*,*E*)-Ethyl 9-(*tert*-butyl)-12-isopropyl-2,2,5,11,14-pentamethyl-4,7,10-trioxo-6-(2-(4-((13-oxo-3,6,9-trioxa-12-thiatetradecyl)oxy)phenyl)propan-2-yl)-3-oxa-5,8,11-triazapentadec-13-en-15-oate.

The title compound was prepared from (*S*)-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-(4-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy) ethoxy)phenyl)-3-methylbutanoic acid (66 mg, 0.065 mmol) following the same procedure described above to give 32 mg (57%) as a clear film after flash chromatography (20–100% EtOAc/Hex) ^{1}H NMR (400 MHz, Chloroform-*d*) δ (ppm) 7.44 (d, *J* = 8.5 Hz, 1.3H), 7.32 (d, *J* = 8.5 Hz, 0.7H), 6.95-6.77 (m, 2H), 6.62 (dd, *J* = 9.2, 1.7 Hz, 1H), 6.09 (d, *J* = 9.1 Hz, 1H), 5.24 (s, 0.7H), 5.13-4.95 (m, 1H), 4.84 (s, 0.3H), 4.69 (d, *J* = 9.6 Hz, 0.3H), 4.60 (d, *J* = 9.0 Hz, 0.7H), 4.19 (q, *J* = 7.1 Hz, 2H), 4.09 (td, *J* = 4.7, 2.4 Hz, 2H), 3.84 (t, *J* = 4.9 Hz, 2H), 3.72 (dd, *J* = 5.7, 3.2 Hz, 2H), 3.70-3.65 (m, 2H), 3.66-3.62 (m, 4H), 3.60 (t, *J* = 6.5 Hz, 2H), 3.09 (t, *J* = 6.5 Hz, 2H), 2.96-2.88 (m, 3H), 2.84 (s, 3H), 2.33 (s, 3H), 1.88 (d, *J* = 3.5 Hz, 3H), 1.49 (s, 2H), 1.43 (d, *J* = 5.5 Hz, 11H), 1.35 (s, 2H), 1.30 (t, *J* = 7.1 Hz, 2H), 0.87 (d, *J* = 6.6 Hz, 3H), 0.80 (d, *J* = 15.9 Hz, 3H), 0.76 (s, 9H). $C_{44}H_{73}N_3O_{11}S$ calcd. $[M+H]^+$ = 852.51 amu; found *m/z* = 852.79. R_f = 0.60 (60% EtOAc/Hex).

Example 3.145: (6*S*,9*S*,12*S*,*E*)-Ethyl 9-(*tert*-butyl)-12-isopropyl-2,2,5,11,14-pentamethyl-4,7,10-trioxo-6-(2-(3-((16-oxo-3,6,9-trioxa-12-thiatetradecyl)oxy)phenyl)propan-2-yl)-3-oxa-5,8,11-triazapentadec-13-en-15-oate.

The title compound was prepared from (*S*)-3-(3-((14-hydroxy-3,6,9,12-tetraoxatetradecyl)oxy)phenyl)-3-methyl-2-(methylamino)butanoic acid (73 mg, 0.080 mmol) following the same procedure described above to give 66 mg (47%) as a clear film after flash chromatography (20-100% EtOAc/Hex). ^{1}H NMR (400 MHz, Chloroform-*d*) δ (ppm) 7.25-6.92 (m, 3H), 6.78-6.70 (m, 1H), 6.62 (d, *J* = 8.9 Hz, 1H), 6.12 (d, *J* = 8.9 Hz, 1H), 5.26 (s, 0.7H), 5.12-4.99 (m, 1H), 4.89 (s, 0.3H), 4.74-4.56 (m, 1H), 4.19 (q, *J* = 7.2 Hz, 1H), 4.16-4.03 (m, 2H), 3.84 (td, *J* = 5.0, 3.2 Hz, 2H), 3.77-3.61 (m, 14H), 3.60 (t, *J* = 6.4 Hz, 2H), 3.09 (t, *J* = 6.5 Hz, 2H), 2.97-2.75 (m, 6H), 2.33 (s, 3H), 1.91-1.83 (m, 3H), 1.52-1.35 (m, 16H), 1.26 (t, *J* = 7.1 Hz, 3H), 0.87 (d, *J* = 6.0 Hz, 3H), 0.81 (d, *J* = 12.9 Hz, 3H), 0.77 (s, 9H). $C_{46}H_{77}N_3O_{12}S$ calcd. $[M+H]^+$ = 896.53 amu; found *m/z* = 896.68. R_f = 0.61 (75% EtOAc/Hex).

Example 3.146: (*S*,*E*)-4-((*S*)-2-((*S*)-3-(4-((14-Mercapto-3,6,9,12-tetraoxatetradecyl)oxy)phenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enoic acid disulfide.

The title compound was prepared by saponification, then TFA promoted Boc removal, according to the exact methods described in Nieman *et al.* from (6*S*,9*S*,12*S*,*E*)-ethyl 9-(*tert*-butyl)-12-

isopropyl-2,2,5,11,14-pentamethyl-4,7,10-trioxo-6-(2-(4-((16-oxo-3,6,9,12-tetraoxa-15-thiaheptadecyl)oxy)phenyl)propan-2-yl)-3-oxa-5,8,11-triazapentadec-13-en-15-oate (26 mg, 0.029 mmol) to afford the title compound (16 mg, 90%) as a clear glass after complete removal of excess TFA. ^{1}H NMR (400 MHz, Methanol-d_4) δ (ppm) 8.43 (d, J = 8.1 Hz, 1H), 7.47 (d, J = 8.5 Hz, 2H), 7.08-6.94 (m, 2H), 6.80 (dq, J = 9.9, 1.5 Hz, 1H), 5.08 (t, J = 10.1 Hz, 1H), 4.94 (d, J = 8.1 Hz, 1H), 4.32 (s, 1H), 4.21-4.12 (m, 2H), 3.93-3.81 (m, 3H), 3.76 (t, J = 6.4 Hz, 2H), 3.76-3.72 (m, 2H), 3.72-3.62 (m, 10H), 3.17 (s, 3H), 2.92 (t, J = 6.4 Hz, 2H), 2.61-2.47 (m, 3H), 2.14-2.00 (m, 1H), 1.94 (d, J = 1.5 Hz, 3H), 1.46 (s, 3H), 1.40 (d, J = 7.7 Hz, 3H), 1.09 (s, 9H), 0.94 (d, J = 5.0 Hz, 3H), 0.92 (d, J = 4.8 Hz, 3H). $C_{74}H_{124}N_6O_{18}S_2$ calcd. $[M+H]^+$ = 1449.85 amu; found m/z = 1450.49.

Example 3.147: (*S*,*E*)-4-((*S*)-2-((S)-3-(4-((14-mercapto-3,6,9,12-tetraoxatetradecyl)oxy)phenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enoic acid.

Compound of Example 3.146 is reduced according to the methods below to produce the subject compound.

Example 3.148: (*S*,*E*)-4-((*S*)-2-((*S*)-3-(4-(2-(2-(2-(2-Mercaptoethoxy)ethoxy)ethoxy)ethoxy)phenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enoic acid disulfide.

The title compound was prepared by saponification, then TFA promoted Boc removal, according to the exact methods described in Nieman *et al.* from (6*S*,9*S*,12*S*,*E*)-ethyl 9-(*tert*-butyl)-12-isopropyl-2,2,5,11,14-pentamethyl-4,7,10-trioxo-6-(2-(4-((13-oxo-3,6,9-trioxa-12-thiatetradecyl)oxy)phenyl)propan-2-yl)-3-oxa-5,8,11-triazapentadec-13-en-15-oate (32 mg, 0.037 mmol) to afford the title compound (29 mg, 86%) as a clear glass after complete removal of excess TFA. ^{1}H NMR (400 MHz, Methanol-d_4) δ (ppm) 8.39 (d, J = 8.2 Hz, 1H), 7.44 (d, J = 8.9 Hz, 2H), 7.01 (d, J = 8.5 Hz, 2H), 6.77 (d, J = 7.9 Hz, 1H), 5.05 (t, J = 10.1 Hz, 1H), 4.92 (d, J = 8.3 Hz, 1H), 4.28 (s, 1H), 4.15 (dd, J = 5.8, 3.4 Hz, 2H), 3.89-3.80 (m, 2H), 3.73 (t, J = 6.4 Hz, 2H), 3.72-3.69 (m, 2H), 3.69-3.60 (m, 6H), 3.14 (s, 3H), 2.89 (t, J = 6.4 Hz, 2H), 2.50 (s, 3H), 2.11-1.97 (m, 1H), 1.91 (d, J = 1.4 Hz, 3H), 1.43 (s, 3H), 1.36 (s, 3H), 1.06 (s, 9H), 0.92-0.87 (m, 6H). $C_{70}H_{118}N_6O_{16}S_2$ calcd. $[M+H]^+$ = 1361.80 amu; found m/z = 1362.26.

Example 3.149: (*S*,*E*)-4-((*S*)-2-((S)-3-(4-(2-(2-(2-(2-mercaptoethoxy)ethoxy)ethoxy)ethoxy)phenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enoic acid.

Compound of Example 3.148 is reduced according to the methods below to produce the subject compound.

Example 3.150: (*S*,*E*)-4-((*S*)-2-((*S*)-3-(3-((14-Mercapto-3,6,9,12-tetraoxatetradecyl)oxy)phenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enoic acid.

The title compound was prepared by saponification, then TFA promoted Boc removal, according to the exact methods described in Nieman *et al.* from (6*S*,9*S*,12*S*,*E*)-ethyl 9-(*tert*-butyl)-12-isopropyl-2,2,5,11,14-pentamethyl-4,7,10-trioxo-6-(2-(3-((16-oxo-3,6,9,12-tetraoxa-15-thiaheptadecyl)oxy)phenyl)propan-2-yl)-3-oxa-5,8,11-triazapentadec-13-en-15-oate (56 mg, 0.029 mmol) to afford the title compound (43 mg, 82%) as an off-white foam after complete removal of excess TFA. ^{1}H NMR (400 MHz, Methanol-d_4) δ (ppm) 8.48 (d, J = 8.3 Hz, 1H), 7.47-7.29 (m, 1H), 7.21-7.04 (m, 1H), 6.95 (t, J = 9.4 Hz, 1H), 6.80 (d, J = 9.7 Hz, 1H), 5.08 (t, J = 10.1 Hz, 1H), 4.97-4.94 (m, 1H), 4.38 (s, 1H), 4.24-4.13 (m, 2H), 3.95-3.82 (m, 2H), 3.80-3.58 (m, 14H), 3.17 (s, 3H), 2.92 (t, J = 6.4 Hz, 2H), 2.53 (s, 3H), 2.11-2.03 (m, 1H), 1.94 (d, J = 1.4 Hz, 3H), 1.47 (s, 3H), 1.40 (s, 3H), 1.09 (s, 9H), 0.93 (dt, J = 11.2, 3.4 Hz, 15H). $C_{74}H_{124}N_6O_{18}S_2$ calcd. $[M+H]^+$ = 1449.85 amu; found *m/z* = 1450.06.

Example 3.151: (*S*,*E*)-4-((*S*)-2-((*S*)-3-(3-((14-mercapto-3,6,9,12-tetraoxatetradecyl)oxy)phenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enoic acid

Compound of Example 3.150 is reduced according to the methods below to produce the subject compound.

Example 3.152: (*S*,*E*)-*N*-(Benzylsulfonyl)-4-((*S*)-2-((*S*)-3-cyclohexyl-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enamide.

The title compound was synthesized from (*S*)-2-(*tert*-butoxycarbonyl(methyl)amino)-3-cyclohexyl-3-methylbutanoic acid as prepared by Zask *et al.* , J. Med. Chem. 2004, 47, (19), 4774-4786 and (*S*,*E*)-4-((*S*)-2-amino-*N*,3,3-trimethylbutanamido)-*N*-(benzylsulfonyl)-2,5-dimethylhex-2-enamide, prepared using General Procedures 15, 16, 10 and 9 by application of General Procedures 11 and 12. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.38 (s, 5H), 6.37 (dd, J = 9.4, 1.7 Hz, 1H), 5.01 (t, J = 10.0 Hz, 1H), 4.91 (s, 1H), 4.75 (s, 2H), 4.01 (s, 1H), 3.10 (s, 3H), 2.66 (s, 3H), 2.05-1.91 (m, 4H), 1.91-1.67 (m, 6H), 1.45-1.28 (m, 3H), 1.29-1.01 (m, 17H), 0.95-0.75 (m, 9H). $C_{34}H_{56}N_4O_5S$ calcd. *m/z* = 632.40 found $[M+H]^+$ = 633.35.

Example 3.153: MC-VC-PABC-3.71.

The title compound was prepared by application of General Procedure 20 and 12 to Boc protected Example 3.58. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.60 (d, J = 8.1 Hz, 2H), 7.56 (d, J = 7.8

Hz, 2H), 7.47 (t, J = 7.6 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 7.33 (d, J = 8.2 Hz, 2H), 7.26 (d, J = 8.0 Hz, 2H), 7.22 (d, J = 7.9 Hz, 2H), 6.81 (s, 2H), 6.37 (d, J = 9.3 Hz, 1H), 5.13-5.01 (m, 3H), 4.96 (s, 1H), 4.70 (s, 2H), 4.56-4.51 (m, 1H), 4.38 (s, 1H), 4.23-4.16 (m, 1H), 3.50 (t, J = 7.1 Hz, 2H), 3.27-3.19 (m, 1H), 3.18-3.04 (m, 4H), 2.52 (s, 3H), 2.30 (t, J = 7.4 Hz, 2H), 2.15-2.05 (m, 1H), 1.96 (s, 3H), 1.98-1.88 (m, 1H), 1.83-1.73 (m, 1H), 1.64 (dq, J = 23.1, 7.3 Hz, 7H), 1.48 (s, 3H), 1.39 (s, 3H), 1.37-1.30 (m, 2H), 1.27 (s, 2H), 1.21 (s, 2H), 1.08 (s, 9H), 1.00 (d, J = 6.7 Hz, 3H), 0.99 (d, J = 6.8 Hz, 3H), 0.91 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.5 Hz, 3H). $C_{66}H_{93}N_{11}O_{13}S$ calcd. m/z = 1279.7 found $[M+H]^+$ = 1281.0.

Example 3.154: MC-VC-PABC-3.76.

The title compound was prepared by application of General Procedures 20 and 12 to Boc protected Example 3.76. $C_{65}H_{91}N_{11}O_{13}S$ calcd. m/z = 1265.7 found $[M+H]^+$ = 1266.7

It is understood to those skilled in the art that it may be possible to carry out the chemical conversions shown in the schemes above with modifications of one or more parameters. As examples, alternate non-nucleophilic solvents may be suitable for the chemistry, such as THF, DMF, Toluene *etc.* Reaction temperatures may be varied. Alternate reagents may be suitable to act as dehydrating or acid-activating agents which are normally used in amide formation reactions, such as pentafluorophenyl esters, NHS esters, EDAC, HBTU, HOBT *etc.*

Example 3.155: Fmoc-Val-Lys(Boc)-OH: (S)-2-((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-methylbutanamido)-6-(tert-butoxycarbonylamino)hexanoic acid.

The title compound was prepared based on the procedure from M. A. Walker, *et al. Bio. Org. Med. Chem. Lett.* **2004**, *14*, 4323–4327 starting with (S)-2,5-dioxopyrrolidin-1-yl 2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-methylbutanoate. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.28 (d, J = 7.8 Hz, 1H), 7.82 (d, J = 7.5 Hz, 2H), 7.69 (t, J = 7.1 Hz, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.33 (td, J = 7.5, 1.2 Hz, 2H), 7.20 (d, J = 8.5 Hz, 1H), 4.49 – 4.36 (m, 3H), 4.26 (t, J = 7.0 Hz, 1H), 3.97 (t, J = 8.0 Hz, 1H), 3.05 – 2.97 (m, 2H), 2.08 (dq, J = 13.3, 6.6 Hz, 1H), 1.93 – 1.84 (m, 1H), 1.81 – 1.66 (m, 1H), 1.54 – 1.43 (m, 4H), 1.40 (s, 9H), 1.01 (d, J = 6.8 Hz, 3H), 0.98 (d, J = 6.8 Hz, 3H). m/z calcd. for $C_{31}H_{41}N_3O_7$ = 567.3 found $[M\text{-Boc}+H^+]^+$ = 468.8.

Example 3.156: Boc-Val-Cit-OH: (*S*)-2-((*S*)-2-(*tert*-Butoxycarbonylamino)-3-methylbutanamido)-5-ureidopentanoic Acid.

The title compound was synthesized according to US2010/0233190 A1 with matching spectroscopic data.

Example 3.157: H-Val-Cit-OH: (*S*)-2-((*S*)-2-Amino-3-methylbutanamido)-5-ureidopentanoic acid.

The title compound was prepared from Boc-VC-OH according to General Procedure 7. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.69 (d, *J* = 7.4 Hz, 1H), 8.21 – 7.97 (m, 3H), 4.24 (td, *J* = 8.2, 4.9 Hz, 1H), 3.97 (s, 0H), 3.63 (dd, *J* = 9.2, 4.0 Hz, 1H), 2.98 (t, *J* = 6.8 Hz, 2H), 2.60 (s, 1H), 2.10 (h, *J* = 6.8 Hz, 1H), 1.85 – 1.69 (m, 1H), 1.61 (dtd, *J* = 14.1, 9.0, 5.6 Hz, 1H), 1.45 (dtd, *J* = 14.7, 8.2, 7.3, 3.7 Hz, 2H), 0.97 (dd, *J* = 6.9, 5.0 Hz, 6H).

Example 3.158: Fmoc-Ala(D)-Phe-Lys(Boc)-OH: (5*R*,8*S*,11*S*)-8-Benzyl-11-(4-(tert-butoxycarbonylamino)butyl)-1-(9H-fluoren-9-yl)-5-methyl-3,6,9-trioxo-2-oxa-4,7,10-triazadodecan-12-oic acid.

The title compound was prepared from Example 2.10 by general procedure 5, followed by treatment with (*R*)-2,5-dioxopyrrolidin-1-yl 2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)propanoate per general procedure 9. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.57 (s, 1H), 8.20 (d, *J* = 7.6 Hz, 1H), 8.12 (d, *J* = 8.8 Hz, 1H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.71 (t, *J* = 6.7 Hz, 2H), 7.48 – 7.37 (m, 3H), 7.33 (t, *J* = 7.4 Hz, 2H), 7.30 – 7.13 (m, 5H), 6.77 (t, *J* = 5.1 Hz, 1H), 4.59 (td, *J* = 10.8, 10.3, 3.5 Hz, 1H), 4.33 – 4.10 (m, 4H), 4.02 (q, *J* = 7.1 Hz, 1H), 3.10 (dd, *J* = 13.8, 2.8 Hz, 1H), 2.94 – 2.87 (m, 2H), 2.79 – 2.67 (m, 1H), 1.75 – 1.70 (m, 1H), 1.62 (s, 1H), 1.37 (s, 4H), 1.36 (s, 9H), 0.96 (d, *J* = 7.1 Hz, 3H). *m/z* calcd. for $C_{31}H_{41}N_3O_7$ = 686.3 found $[M+Na^+]^+$ = 709.9.

Example 3.159: Fmoc-Phe(D)-Phe-Lys-OH: (5*R*,8*S*,11*S*)-5,8-dibenzyl-11-(4-(tert-butoxycarbonylamino)butyl)-1-(9H-fluoren-9-yl)-3,6,9-trioxo-2-oxa-4,7,10-triazadodecan-12-oic acid.

The title compound was prepared from Example 2.10 by general procedure 5, followed by treatment with (*R*)-2,5-dioxopyrrolidin-1-yl 2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-3-phenylpropanoate per general procedure 9. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.59 (s, 1H), 8.39 (d, *J* = 8.7 Hz, 1H), 8.31 (d, *J* = 7.6 Hz, 1H), 7.88 (d, *J* = 7.5 Hz, 2H), 7.62 (t, *J* = 8.2 Hz, 2H), 7.47 (d, *J* = 8.7 Hz, 1H), 7.41 (t, *J* = 7.1 Hz, 2H), 7.35 – 7.10 (m, 12H), 6.77 (t, *J* = 5.7 Hz, 1H), 4.73 – 4.62 (m, 1H), 4.28 – 4.03 (m, 5H), 3.09 (dd, *J* = 13.7, 3.8 Hz, 1H), 2.93 – 2.87 (m, 2H), 2.74 (dd, *J* = 13.7, 10.4 Hz, 1H), 2.58 (dd, *J* = 13.8, 3.4 Hz, 1H), 2.48 – 2.35 (m, 1H), 1.84 – 1.68 (m, 1H), 1.68 – 1.55 (m, 1H), 1.40 – 1.33 (m, 13H). *m/z* calcd. for $C_{31}H_{41}N_3O_7$ = 762.4 found $[M+Na^+]^+$ = 785.9.

Example 3.160: (*S*,*E*)-N-(4-(1-((14*S*,17*S*)-1-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)cyclopropyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound R)

Step 1: (S,E)-N-(4-(1-((S)-2-((S)-2-amino-3-methylbutanamido)-5-ureidopentanamido)cyclopropyl)phenylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (R-1) was synthesized from Compound Q-2 according to General Procedure 7. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.97 – 7.90 (m, 2H), 7.59 – 7.51 (m, 2H), 7.47 (dd, J = 8.5, 6.9 Hz, 2H), 7.44 – 7.34 (m, 3H), 6.46 (dd, J = 9.4, 1.7 Hz, 1H), 5.02 (t, J = 10.0 Hz, 1H), 4.93 (s, 1H), 4.43 (dd, J = 8.6, 5.8 Hz, 1H), 4.35 (s, 1H), 3.71 (d, J = 5.7 Hz, 1H), 3.23 – 3.09 (m, 5H), 2.51 (s, 3H), 2.22 (dt, J = 13.4, 6.7 Hz, 1H), 2.04 (q, J = 8.8, 7.8 Hz, 1H), 1.89 – 1.68 (m, 4H), 1.58 (dq, J = 14.5, 8.7, 8.3 Hz, 2H), 1.48 (s, 4H), 1.36 (d, J = 14.3 Hz, 5H), 1.15 – 0.99 (m, 16H), 0.90 (dd, J = 6.6, 3.4 Hz, 6H). *m/z* calcd. for $C_{47}H_{73}N_9O_8S$ = 923.53. Found $[M+H]_+$ = 924.8.

Step 2: (*S*,*E*)-*N*-(4-(1-((14*S*,17*S*)-1-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)cyclopropyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide was synthesized from R-1 and MT-NHS according to General Procedure 6 prior to purification by preparative HPLC-MS. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.99 – 7.91 (m, 2H), 7.60 – 7.52 (m, 2H), 7.48 (t, J = 7.7 Hz, 2H), 7.44 – 7.31 (m, 3H), 6.84 (s, 2H), 6.45 (dd, J = 9.3, 1.7 Hz, 1H), 5.00 (t, J = 10.0 Hz, 1H), 4.94 (s, 1H), 4.35 (d, J = 5.3 Hz, 2H), 4.21 (d, J = 6.9 Hz, 1H), 3.81 – 3.67 (m, 4H), 3.67 – 3.54 (m, 10H), 3.25 – 3.05 (m, 5H), 2.64 – 2.47 (m, 5H), 2.20 – 1.99 (m, 2H), 1.85 (d, J = 1.3 Hz, 4H), 1.73 (dq, J = 9.5, 4.5 Hz, 1H), 1.66 – 1.28 (m, 11H), 1.12 – 0.94 (m, 16H), 0.90 (dd, J = 6.6, 4.9 Hz, 6H). *m/z* calcd. for $C_{60}H_{90}N_{10}O_{14}S$ = 1206.64. Found $[M+H]^+$ = 1207.9.

Example 3.161: (*R*)-*N*-((2*S*,3*S*)-1-(((*S*,*E*)-6-(4-((14*S*,17*S*)-1-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3-methyl-1-oxopentan-2-yl)-1-methylpiperidine-2-carboxamide (Compound S).

Step 1: (*S*,*E*)-Ethyl 4-(*tert*-Butoxycarbonyl(methyl)amino)-2,5-dimethylhex-2-enoate, Boc-ICD-OEt (S-1) was synthesized from (*S*,*E*)-ethyl 2,5-dimethyl-4-(methylamino)hex-2-enoate (synthesized according to US 7,579,323 B1) and Boc-Isoleucine-OH and using General Procedure 4. NMR provided for a sample treated with TFA to remove the Boc group and resolve rotamers in the spectrum. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.68 (dd, J = 9.5, 1.8 Hz, 1H), 5.33 (s, 0H), 4.97 (t, J

= 9.9 Hz, 1H), 4.36 (d, *J* = 4.1 Hz, 1H), 4.25 (q, *J* = 7.1 Hz, 2H), 3.56 (s, 1H), 2.96 (s, 3H), 2.07 – 1.83 (m, 5H), 1.53 (s, 1H), 1.34 (t, *J* = 7.1 Hz, 3H), 1.12 (d, *J* = 7.0 Hz, 3H), 1.00 – 0.83 (m, 9H).

Step 2: (*S*,*E*)-4-((2*S*,3*R*)-2-(*tert*-Butoxycarbonylamino)-*N*,3-dimethylpentanamido)-2,5-dimethylhex-2-enoic acid (S-2) was generated from Boc-ICD-OEt using General Procedure 11. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.79 (dd, *J* = 9.3, 1.7 Hz, 1H), 5.28 (d, *J* = 9.7 Hz, 1H), 5.11 (dd, *J* = 10.6, 9.2 Hz, 1H), 4.46 – 4.34 (m, 1H), 3.01 (s, 3H), 1.94 (s, *J* = 1.5 Hz, 4H), 1.77 – 1.54 (m, 2H), 1.44 (s, 9H), 1.14 (dt, *J* = 15.8, 8.0 Hz, 1H), 0.97 – 0.81 (m, 12H).

Step 3: (*S*,*E*)-4-((2*S*,3*S*)-*N*,3-Dimethyl-2-((*R*)-1-methylpiperidine-2-carboxamido)pentanamido)-2,5-dimethylhex-2-enoic acid (S-3) was synthesized from Compound S-1 according to General Procedure 7 and reacting the liberated amine with D-(*N*-methyl)-pipecolic acid using General Procedure 4. Finally, the C-terminal carboxylate was liberated using General Procedure 11 prior to purification by preparative scale HPLC. ^{1}H NMR (400 MHz, Methanol-d_4) δ 6.77 (dd, *J* = 9.5, 1.4 Hz, 1H), 5.04 (t, *J* = 10.1 Hz, 1H), 4.65 – 4.56 (m, 1H), 3.79 – 3.69 (m, 1H), 3.54 – 3.45 (m, 1H), 3.12 (s, 3H), 3.10 – 3.06 (m, 1H), 2.76 (s, 3H), 2.21 – 2.10 (m, 1H), 2.08 – 2.00 (m, 1H), 2.01 – 1.92 (m, 2H), 1.90 (d, *J* = 1.5 Hz, 3H), 1.88 – 1.72 (m, 3H), 1.69 – 1.52 (m, 2H), 1.31 – 1.16 (m, 1H), 0.98 – 0.86 (m, 12H). $C_{22}H_{39}N_3O_4$ calcd. *m/z* = 409.29 found $[M+H]^+$ =410.91

Step 4: (*S*,*E*)-4-((2*S*,3*S*)-2-Amino-*N*,3-dimethylpentanamido)-2,5-dimethyl-*N*-(4-(2,2,2-trifluoroacetamido)phenylsulfonyl)hex-2-enamide (S-4) was prepared from Compound S-2 according to General Procedure 11, followed by *N*-acyl sulfonamide generation with 2,2,2-trifluoro-*N*-(4-sulfamoylphenyl)acetamide according to General Procedure 1, followed by General Procedure 7. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.00 – 7.85 (m, 2H), 7.76 (d, *J* = 8.8 Hz, 2H), 6.39 (dd, *J* = 9.2, 1.8 Hz, 1H), 4.45 – 4.30 (m, 1H), 4.14 (d, *J* = 4.1 Hz, 1H), 2.82 (s, 3H), 2.08 – 1.91 (m, 1H), 1.67 (s, *J* = 1.5 Hz, 3H), 1.41-1.35 (m, *J* = 13.3, 7.6, 3.2 Hz, 1H), 1.10 – 0.88 (m, 4H), 0.77 (ddd, *J* = 17.2, 9.0, 5.4 Hz, 9H).

Step 5: (*R*)-*N*-((2*S*,3*S*)-1-(((*S*,*E*)-2,5-Dimethyl-6-oxo-6-(4-(2,2,2-trifluoroacetamido)phenylsulfonamido)hex-4-en-3-yl)(methyl)amino)-3-methyl-1-oxopentan-2-yl)-1-methylpiperidine-2-carboxamide (S-5) was prepared from Compound S-4 and *N*-methyl-D-pipecolic acid according to General Procedure 4. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.97 (d, 2H), 7.77 (d, 2H), 7.67 (d, *J* = 8.6 Hz, 0H), 6.60 (d, *J* = 9.2 Hz, 1H), 4.96 (t, *J* = 9.9 Hz, 1H), 4.61 (d, *J* = 8.8 Hz, 1H), 3.75 (hept, *J* = 6.6 Hz, 1H), 3.19-3.10 (m, 1H), 3.06 (s, 3H), 2.45 (s, 2H), 2.39 (s, 3H), 2.01-1.88 (m, 3H), 1.84 (d, *J* = 1.4 Hz, 3H), 1.78-1.54 (m, 5H), 1.25-1.13 (m, 1H), 0.92 (s, 1H), 0.91-0.86 (m, 8H), 0.83 (d, *J* = 6.6 Hz, 3H). $C_{30}H_{44}F_3N_5O_6S$ calcd. *m/z* = 659.30 found $[M+H]^+$ = 660.88

Step 6: (*R*)-*N*-((2*S*,3*S*)-1-(((*S*,*E*)-6-(4-Aminophenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3-methyl-1-oxopentan-2-yl)-1-methylpiperidine-2-carboxamide (L-6) was prepared from Compound S-5 according to General Procedure 3. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.72 (d, 2H), 6.69 (d, 2H), 6.42 (dd, *J* = 9.2, 1.7 Hz, 1H), 4.61-4.55 (m, 1H), 3.72 (dd, *J* = 12.2, 3.2

Hz, 1H), 3.52-3.44 (m, 1H), 3.37 (s, 3H), 3.12 (s, 3H), 3.09-3.03 (m, 1H), 2.71 (s, 3H), 2.20-1.92 (m, 3H), 1.84 (d, J = 1.4 Hz, 3H), 1.80-1.72 (m, 2H), 1.67-1.53 (m, 2H), 1.29-1.16 (m, 1H), 0.96-0.85 (m, 12H). $C_{28}H_{45}N_5O_5S$ calcd. m/z = 563.31 found $[M+H]^+$ = 564.93.

Step 7: (*R*)-*N*-((2*S*,3*S*)-1-(((*S*,*E*)-6-(4-((14*S*,17*S*)-1-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3-methyl-1-oxopentan-2-yl)-1-methylpiperidine-2-carboxamide was prepared from Compound S-6 and MT-Val-Cit-OH according to General Procedure 10. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.00 (d, 2H), 7.88 (d, 2H), 6.83 (s, 2H), 6.46 (dd, J = 9.1, 1.6 Hz, 1H), 4.57 (d, J = 8.3 Hz, 1H), 4.55-4.52 (m, 1H), 4.22 (d, J = 6.9 Hz, 1H), 3.80-3.73 (m, 3H), 3.73-3.66 (m, 2H), 3.66-3.60 (m, 2H), 3.58 (d, J = 2.2 Hz, 8H), 3.52-3.43 (m, 1H), 3.26-3.19 (m, 1H), 3.17-3.13 (m, 2H), 3.12 (s, 4H), 2.71 (s, 3H), 2.61-2.55 (m, 2H), 2.21-2.01 (m, 3H), 2.00-1.88 (m, 3H), 1.83 (d, J = 1.4 Hz, 3H), 1.81-1.71 (m, 4H), 1.68-1.52 (m, 4H), 1.29-1.14 (m, 1H), 1.01 (t, J = 6.8 Hz, 6H), 0.94-0.86 (m, 12H). $C_{52}H_{82}N_{10}O_{14}S$ calcd. m/z =1102.57 found $[M+H]^+$ = 1104.22

Example 3.162: (*R*)-*N*-((*S*)-1-(((*S*,*E*)-6-(4-((14*S*,17*S*)-1-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)-1-methylpiperidine-2-carboxamide (Compound T).

Step 1: (*S*,*E*)-2,5-Dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*R*)-1-methylpiperidine-2-carboxamido)butanamido)hex-2-enoic acid (T-1) was prepared from (*S*,*E*)-ethyl 4-((*S*)-2-amino-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enoate (synthesized according to US 7,579,323 B1) and D-*N*-methyl-pipecolic acid according to General Procedures 4 and 11. ^{1}H NMR (400 MHz, Methanol-d_4) δ 6.60 (dd, J = 9.4, 1.7 Hz, 1H), 5.04 (t, J = 10.0 Hz, 1H), 4.77 (s, 1H), 4.62 (s, 1H), 3.30-3.23 (m, 1H), 3.10 (s, 3H), 2.68 (t, J = 12.2 Hz, 1H), 2.52 (s, 3H), 2.04 (s, 1H), 2.02-1.93 (m, 2H), 1.90 (d, J = 1.4 Hz, 3H), 1.88-1.79 (m, 1H), 1.77-1.62 (m, 2H), 1.56-1.43 (m, 1H), 1.04 (s, 9H), 0.92 (d, J = 6.6 Hz, 3H), 0.85 (d, J = 6.6 Hz, 3H). $C_{22}H_{39}N_3O_4$ calcd. m/z = 409.29 found $[M+H]^+$ = 410.92

Step 2: (*R*)-*N*-((*S*)-1-(((*S*,*E*)-2,5-Dimethyl-6-oxo-6-(4-(2,2,2-trifluoroacetamido)phenylsulfonamido)hex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)-1-methylpiperidine-2-carboxamide (T-2) was prepared from Compound T-1 and 2,2,2-trifluoro-*N*-(4-sulfamoylphenyl)acetamide using General Procedure 2. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.08 (d, J = 8.8 Hz, 2H), 7.92 (d, J = 8.9 Hz, 2H), 6.47 (d, J = 9.0 Hz, 1H), 5.01-4.92 (m, 1H), 4.70 (s, 1H), 3.82 (d, J = 12.3 Hz, 1H), 3.53-3.43 (m, 1H), 3.13 (s, 3H), 2.72 (s, 3H), 2.22-1.90 (m, 4H), 1.85 (d, J = 1.4 Hz, 5H), 1.60 (m, 1H), 1.40-1.22 (m, 4H), 1.03 (s, 9H), 0.89 (dd, J = 17.1, 6.5 Hz, 6H). $C_{30}H_{44}F_3N_5O_6S$ calcd. m/z = 659.76 found $[M+H]^+$ = 660.95

Step 3: (*R*)-*N*-((*S*)-1-(((*S*,*E*)-6-(4-Aminophenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)-1-methylpiperidine-2-carboxamide (T-3) was prepared from Compound T-2 according to General Procedure 3. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.76-7.66 (m, 2H), 6.74-6.64 (m, 2H), 6.42 (dd, J = 8.9, 1.7 Hz, 1H), 4.94 (m, 1H), 4.70 (s, 1H), 3.82 (dd, J = 12.2, 3.1 Hz, 1H), 3.54-3.42 (m, 1H), 3.13 (s, 4H), 2.70 (s, 3H), 2.16 (d, J = 14.6 Hz, 1H), 2.11-2.01 (m, 1H), 1.96 (d, J = 12.9 Hz, 2H), 1.89-1.51 (m, 6H), 1.03 (s, 9H), 0.89 (dd, J = 16.3, 6.5 Hz, 6H). $C_{28}H_{45}N_5O_5S$ calcd. m/z = 563.31 found $[M+H]^+$ = 564.93.

Step 4: (*R*)-*N*-((*S*)-1-(((*S*,*E*)-6-(4-((14*S*,17*S*)-1-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)-1-methylpiperidine-2-carboxamide was prepared from Compound T-3 and MT-Val-Cit-OH according to General Procedure 10. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.00 (d, J = 8.9 Hz, 2H), 7.88 (d, J = 8.7 Hz, 2H), 6.83 (s, 2H), 6.46 (d, J = 9.1 Hz, 1H), 4.96-4.91 (m, 1H), 4.72-4.68 (m, 1H), 4.58-4.51 (m, 1H), 4.22 (t, J = 7.2 Hz, 1H), 3.83-3.73 (m, 3H), 3.72-3.67 (m, 2H), 3.65-3.61 (m, 2H), 3.61-3.55 (m, 8H), 3.52-3.46 (m, 1H), 3.27-3.19 (m, 1H), 3.13 (s, 3H), 3.09-3.03 (m, 1H), 2.69 (s, 3H), 2.58 (t, J = 6.0 Hz, 2H), 2.19-2.01 (m, 4H), 2.00-1.90 (m, 3H), 1.84 (d, J = 1.4 Hz, 3H), 1.83-1.72 (m, 3H), 1.61 (d, J = 9.0 Hz, 3H), 1.03 (s, 11H), 1.00 (d, J = 6.8 Hz, 4H), 0.91 (d, J = 6.5 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H). $C_{52}H_{82}N_{10}O_{14}S$ calcd. m/z = 1102.57 found $[M+H]^+$ = 1104.30

Example 3.163: (*R*)-*N*-((*S*)-1-(((*S*,*E*)-6-(4-((14*S*,17*S*)-1-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)-1-isopropylpiperidine-2-carboxamide (Compound U).

Step 1: (*R*)-*N*-((*S*)-1-(((*S*,*E*)-2,5-Dimethyl-6-oxo-6-(4-(2,2,2-trifluoroacetamido)phenylsulfonamido)hex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)-1-isopropylpiperidine-2-carboxamide (U-1) was prepared from (*S*,*E*)-4-((*S*)-2-((*R*)-1-isopropylpiperidine-2-carboxamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enoic acid (prepared according to US 2012/0309938 A1) and 2,2,2-trifluoro-*N*-(4-sulfamoylphenyl)acetamide using General Procedure 3. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.00 (d, J = 8.8 Hz, 2H), 7.83 (d, J = 8.8 Hz, 2H), 6.56 (d, J = 9.1 Hz, 1H), 4.69 (s, 1H), 4.12 (dd, J = 11.6, 3.3 Hz, 1H), 3.95 (hept, J = 6.2 Hz, 1H), 3.54-3.41 (m, 2H), 3.37 (s, 3H), 3.08 (s, 3H), 3.04-2.89 (m, 1H), 2.13 (dd, J = 17.2, 6.4 Hz, 1H), 2.00-1.88 (m, 4H), 1.84 (d, J = 1.5 Hz, 4H), 1.71-1.52 (m, 1H), 1.29 (dd, J = 28.0, 6.7 Hz, 8H), 1.17 (d, J = 6.1 Hz, 6H), 1.01 (s, 10H), 0.86 (dd, J = 28.2, 6.5 Hz, 7H). $C_{32}H_{48}F_3N_5O_6S$ calcd. m/z = 687.33 found $[M+H]^+$ = 688.9.

Step 2: (*R*)-*N*-((*S*)-1-(((*S*,*E*)-6-(4-Aminophenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)-1-isopropylpiperidine-2-carboxamide (U-2) was

prepared from Compound U-1 according to General Procedure 3. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.75-7.62 (m, 2H), 6.74-6.62 (m, 2H), 6.59-6.35 (m, 1H), 4.70 (s, 1H), 4.09 (dd, J = 11.7, 3.3 Hz, 1H), 3.52-3.38 (m, 2H), 3.10 (s, 3H), 3.02-2.87 (m, 1H), 2.12 (d, J = 11.9 Hz, 1H), 2.06-1.73 (m, 11H), 1.70-1.50 (m, 1H), 1.28 (dd, J = 28.8, 6.7 Hz, 6H), 1.02 (s, 9H), 0.87 (dd, J = 27.7, 6.5 Hz, 6H). $C_{30}H_{49}N_5O_5S$ calcd. m/z = 591.35 found $[M+H]^+$ = 593.0.

Step 3: *tert*-Butyl (*S*)-1-((*S*)-1-(4-(*N*-((*S*,*E*)-4-((*S*)-2-((*R*)-1-Isopropylpiperidine-2-carboxamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enoyl)sulfamoyl)phenylamino)-1-oxo-5-ureidopentan-2-ylamino)-3-methyl-1-oxobutan-2-ylcarbamate (U-3) was synthesized from Compound U-2 and Boc-Val-Cit-OH according to General Procedure 10. $C_{46}H_{77}N_9O_{10}S$ calcd. m/z = 947.55 found $[M+H]^+$ = 949.2.

Step 4: (*R*)-*N*-((*S*)-1-(((*S*,*E*)-6-(4-((14*S*,17*S*)-1-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)phenylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)-1-isopropylpiperidine-2-carboxamide was prepared from Compound U-3 and MT-NHS according to General Procedures 7 and 6 and purified by preparative HPLC-MS. $C_{54}H_{86}N_{10}O_{14}S$ calcd. m/z = 1130.60 found $[M+H]^+$ = 1132.5.

Example 3.164: (*S*)-*N*-(4-((*N*-((2*R*,3*R*)-3-((*S*)-1-((3*R*,4*S*,5*R*)-4-((*S*)-2-((*S*)-2-(Dimethylamino)-3-methylbutanamido)-*N*,3-dimethylbutanamido)-3-methoxy-5-methylheptanoyl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanoyl)sulfamoyl)methyl)phenyl)-2-((*S*)-1-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12-oxo-3,6,9-trioxa-13-azapentadecanamido)-5-ureidopentanamide (Compound W).

Step 1: *tert*-Butyl (*S*)-1-(((3*R*,4*S*,5*R*)-3-Methoxy-1-((*S*)-2-((1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-((4-(2,2,2-trifluoroacetamido)phenyl)methylsulfonamido)propyl)pyrrolidin-1-yl)-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-ylcarbamate (W-1) was prepared from commercially available Boc-Val-Dil-Dap-OH and 2,2,2-trifluoro-*N*-(4-(sulfamoylmethyl)phenyl)acetamide through General Procedure 2. $C_{38}H_{60}F_3N_5O_{10}S$ calcd. m/z = 835.40 found $[M+H]^+$ = 836.7.

Step 2: (*S*)-2-((*S*)-2-(Dimethylamino)-3-methylbutanamido)-*N*-((3*R*,4*S*,5*R*)-3-methoxy-1-((*S*)-2-((1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-((4-(2,2,2-trifluoroacetamido)phenyl)methylsulfonamido)propyl)pyrrolidin-1-yl)-5-methyl-1-oxoheptan-4-yl)-*N*,3-dimethylbutanamide (W-2) was prepared from Compound W-1 and *N*,*N*-dimethylvaline according to General Procedure 4. $C_{40}H_{65}F_3N_6O_9S$ calcd. m/z = 862.45 found $[M+H]^+$ = 863.2.

Step 3: (*S*)-*N*-((3*R*,4*S*,5*R*)-1-((*S*)-2-((1*R*,2*R*)-3-((4-Aminophenyl)methylsulfonamido)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)-2-((*S*)-2-(dimethylamino)-3-methylbutanamido)-*N*,3-dimethylbutanamide (W-3) was prepared from

Compound W-2 by following General Procedure 3. $C_{38}H_{66}N_6O_8S$ calcd. *m/z* = 766.47 found [M-$C_7H_8O_2S$+H]$^+$ = 599.0 (Quinone methide fragmentation and loss of 4-aminobenzylsulfonate).

Step 4: (*S*)-*N*-(4-((*N*-((2*R*,3*R*)-3-((*S*)-1-((3*R*,4*S*,5*R*)-4-((*S*)-2-((*S*)-2-(Dimethylamino)-3-methylbutanamido)-*N*,3-dimethylbutanamido)-3-methoxy-5-methylheptanoyl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanoyl)sulfamoyl)methyl)phenyl)-2-((*S*)-1-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12-oxo-3,6,9-trioxa-13-azapentadecanamido)-5-ureidopentanamide. was synthesized using General Procedure 10 from MT-VAL-CIT-OH and Compound W-3 and purified by preparative HPLC chromatography. $C_{61}H_{101}N_{11}O_{17}S$ calcd. *m/z* = 1305.73 found [M+H]$^+$ = 1306.9.

Example 3.165: (*S*)-*N*-(4-(*N*-((*S*)-2-((2*R*,3*R*)-3-((*S*)-1-((3*R*,4*S*,5*R*)-4-((*S*)-2-((*S*)-2-(Dimethylamino)-3-methylbutanamido)-*N*,3-dimethylbutanamido)-3-methoxy-5-methylheptanoyl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanamido)-3-phenylpropanoyl)sulfamoyl)phenyl)-2-((*S*)-1-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12-oxo-3,6,9-trioxa-13-azapentadecanamido)-5-ureidopentanamide (Compound X).

Step 1: (*S*)-2-Amino-3-phenyl-*N*-(4-(2,2,2-trifluoroacetamido)phenylsulfonyl)propanamide (X-1) was prepared from Boc-phenylalanine and 2,2,2-trifluoro-*N*-(4-sulfamoylphenyl)acetamide according to General Procedures 2 and 7. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.42 (s, 1H), 7.84 (d, *J* = 8.7 Hz, 2H), 7.73-7.64 (m, 1H), 7.69 (d, *J* = 8.7 Hz, 2H), 7.24-7.14 (m, 3H), 7.13-7.06 (m, 2H), 3.65-3.60 (m, 1H), 3.06 (dd, *J* = 14.2, 5.1 Hz, 1H), 2.91 (dd, *J* = 14.1, 7.1 Hz, 1H). $C_{17}H_{16}F_3N_3O_4S$ calcd. *m/z* = 415.08 found [M+H]$^+$ = 416.5.

Step 2: *tert*-Butyl (*S*)-1-(((3*R*,4*S*,5*R*)-3-Methoxy-1-((*S*)-2-((1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-((*S*)-1-oxo-3-phenyl-1-(4-(2,2,2-trifluoroacetamido)phenylsulfonamido)propan-2-ylamino)propyl)pyrrolidin-1-yl)-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-ylcarbamate (X-2) was synthesized from commercially available Boc-Val-Dip-Dap-OH (0.07 g) and Compound X-1 using General Procedure 4. $C_{46}H_{67}F_3N_6O_{11}S$ calcd. *m/z* = 968.45 found [M+Na]$^+$ = 992.1.

Step 3: (*S*)-2-((*S*)-2-(Dimethylamino)-3-methylbutanamido)-*N*-((3*R*,4*S*,5*R*)-3-methoxy-1-((*S*)-2-((1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-((*S*)-1-oxo-3-phenyl-1-(4-(2,2,2-trifluoroacetamido)phenylsulfonamido)propan-2-ylamino)propyl)pyrrolidin-1-yl)-5-methyl-1-oxoheptan-4-yl)-*N*,3-dimethylbutanamide (X-3) was prepared from Compound X-2 (110 mg) and *N*,*N*-dimethyl valine using General Procedures 7 and 4. $C_{48}H_{72}F_3N_7O_{10}S$ calcd. *m/z* = 995.50 found [M+H]$^+$ 997.3.

Step 4: (*S*)-*N*-((3*R*,4*S*,5*R*)-1-((*S*)-2-((1*R*,2*R*)-3-((*S*)-1-(4-Aminophenylsulfonamido)-1-oxo-3-phenylpropan-2-ylamino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)-2-((*S*)-2-(dimethylamino)-3-methylbutanamido)-*N*,3-dimethylbutanamide (X-4) was

prepared from Compound X-3 (100 mg) using General Procedure 3. $C_{46}H_{73}N_7O_9S$ calcd. *m/z* = 899.52 found $[M+H]^+$ 901.3.

Step 5: (*S*)-*N*-(4-(*N*-((*S*)-2-((2*R*,3*R*)-3-((*S*)-1-((3*R*,4*S*,5*R*)-4-((*S*)-2-((*S*)-2-(Dimethylamino)-3-methylbutanamido)-*N*,3-dimethylbutanamido)-3-methoxy-5-methylheptanoyl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanamido)-3-phenylpropanoyl)sulfamoyl)phenyl)-2-((*S*)-1-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12-oxo-3,6,9-trioxa-13-azapentadecanamido)-5-ureidopentanamide was prepared from Compound X-4 (25 mg) and MT-Val-Cit-OH (63 mg) using General Procedure 10. $C_{70}H_{110}N_{12}O_{18}S$ calcd. *m/z* = 1438.8 amu; found $[M+H]^+$ = 1440.2, $[(M+2H)/2]^{2+}$ = 720.5.

Example 3.166: (*S*)-*N*-((3*R*,4*S*,5*R*)-1-((*S*)-2-((1*R*,2*R*)-3-((*S*)-1-(4-Amino phenylmethylsulfonamido)-1-oxo-3-phenylpropan-2-ylamino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)-2-((*S*)-2-(dimethylamino)-3-methylbutanamido)-*N*,3-dimethylbutanamide (Compound Y)

Step 1: (*S*)-2-Amino-3-phenyl-*N*-(4-(2,2,2-trifluoroacetamido)benzylsulfonyl)propanamide (Y-1) was prepared from Boc-phenylalanine and 2,2,2-trifluoro-*N*-(4-sulfamoylphenyl)acetamide according to General Procedures 9 and 7 (*S*)-*tert*-butyl 1-oxo-3-phenyl-1-(phenylmethylsulfonamido)propan-2-ylcarbamate ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.76-7.71 (m, 2H), 7.58 (d, *J* = 8.4 Hz, 2H), 7.36-7.21 (m, 8H), 4.34 (d, *J* = 13.1 Hz, 1H), 4.30 (d, *J* = 13.1 Hz, 1H), 3.62 (dd, *J* = 8.2, 4.6 Hz, 1H), 3.21-3.09 (m, 1H), 2.89 (dd, *J* = 14.3, 8.3 Hz, 1H). $C_{18}H_{18}F_3N_3O_4S$ calcd. *m/z* = 429.10 found $[M+H]^+$ = 430.7.

Step 2: *tert*-Butyl (*S*)-1-(((3*R*,4*S*,5*R*)-3-methoxy-1-((*S*)-2-((1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-((*S*)-1-oxo-3-phenyl-1-(4-(2,2,2-trifluoroacetamido)phenylmethylsulfonamido)propan-2-ylamino)propyl)pyrrolidin-1-yl)-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-ylcarbamate (Y-2) was prepared from commercially available Boc-Val-Dil-Dap-OH and Compound Y-1 by following General Procedure 4. $C_{47}H_{69}F_3N_6O_{11}S$ calcd. *m/z* =982.47 found $[M+Na]^+$ = 1006.2.

Step 3: (*S*)-2-((*S*)-2-(Dimethylamino)-3-methylbutanamido)-*N*-((3*R*,4*S*,5*R*)-3-methoxy-1-((*S*)-2-((1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-((*S*)-1-oxo-3-phenyl-1-(4-(2,2,2-trifluoroacetamido)phenylmethylsulfonamido)propan-2-ylamino)propyl)pyrrolidin-1-yl)-5-methyl-1-oxoheptan-4-yl)-*N*,3-dimethylbutanamide (Y-3) was prepared from Compound Y-2 and *N*,*N*-dimethylvaline according to General Procedures 7 and 4. $C_{49}H_{74}F_3N_7O_{10}S$ calcd. *m/z* =1009.52 found $[M+H]^+$ = 1011.0.

Step 4: (*S*)-*N*-((3*R*,4*S*,5*R*)-1-((*S*)-2-((1*R*,2*R*)-3-((*S*)-1-(4-Aminophenylmethylsulfonamido)-1-oxo-3-phenylpropan-2-ylamino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)-2-((*S*)-2-(dimethylamino)-3-methylbutanamido)-*N*,3-dimethylbutanamide (Y-4) was prepared from Compound Y-3 according to General Procedure 3. $C_{47}H_{75}N_7O_9S$ calcd. *m/z* =

913.53 found [M-$C_7H_8O_2S$+Na]$^+$ = 768.1 (Quinone methide fragmentation and loss of 4-aminobenzylsulfonate).

Step 5: (*S*)-*N*-((3*R*,4*S*,5*R*)-1-((*S*)-2-((1*R*,2*R*)-3-((*S*)-1-(4-Amino phenylmethylsulfonamido)-1-oxo-3-phenylpropan-2-ylamino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)-2-((*S*)-2-(dimethylamino)-3-methylbutanamido)-*N*,3-dimethylbutanamide was prepared from Compound Y-4 and MT-Val-Cit-OH according to General Procedure 10, followed by purification by preparative HPLC. $C_{71}H_{112}N_{12}O_{18}S$ calcd. *m/z* = 1452.8 found [M+H$^+$]$^+$ = 1454.6.

Example 3.167: (*S*,*E*)-*N*-(4-((14*R*,17*R*)-1-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)-2,3-dimethylphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound Z)

Step 1: *N*-(2,3-Dimethyl-4-sulfamoylphenyl)-2,2,2-trifluoroacetamide (Z-1) synthesized from 2,3-dimethylaniline according to General Procedure 8. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.25 (s, 1H), 7.79 (d, *J* = 8.5 Hz, 1H), 7.48 (s, 2H), 7.29 (d, *J* = 8.5 Hz, 1H), 2.55 (s, 3H), 2.14 (s, 3H).

Step 2: (*S*,*E*)-*N*-(4-Amino-2,3-dimethylphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Z-2) synthesized from Boc-HTI-286-OH and Compound Z-1 using General Procedures 2, 3 and 7. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.75 (d, *J* = 8.8 Hz, 1H), 7.55 (d, *J* = 7.9 Hz, 2H), 7.47 (t, *J* = 7.7 Hz, 2H), 7.37 (t, *J* = 6.9 Hz, 1H), 6.63 (d, *J* = 8.8 Hz, 1H), 6.46 (d, *J* = 9.7 Hz, 1H), 5.00 (t, *J* = 10.0 Hz, 1H), 4.93 (s, 1H), 4.32 (s, 1H), 3.17 (s, 3H), 2.54 (s, 3H), 2.49 (s, 3H), 2.09 (s, 3H), 2.08-2.02 (m, 1H), 1.87 (d, *J* = 1.4 Hz, 3H), 1.47 (s, 3H), 1.37 (s, 3H), 1.07 (s, 9H), 0.92 (dd, *J* = 6.8, 6.5 Hz, 6H). $C_{35}H_{53}N_5O_5S$ calcd. *m/z* = 655.38 found [M+H]$^+$ = 656.4.

Step 3: (*S*,*E*)-*N*-(4-((14*R*,17*R*)-1-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)-2,3-dimethylphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide synthesized from Compound Z-2 and MT-NHS according to General Procedure 6. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.01 (dd, *J* = 11.0, 8.2 Hz, 2H), 7.60-7.51 (m, 2H), 7.47 (dd, *J* = 8.5, 6.8 Hz, 3H), 7.41-7.31 (m, 1H), 6.83 (s, 2H), 6.50 (dd, *J* = 9.5, 1.8 Hz, 1H), 5.01 (t, *J* = 10.0 Hz, 1H), 4.93 (t, *J* = 4.1 Hz, 1H), 4.60 (m, 1H), 4.36 (s, 1H), 4.30-4.17 (m, 1H), 3.80-3.67 (m, 4H), 3.64 (td, *J* = 5.5, 1.2 Hz, 2H), 3.60 (d, *J* = 3.2 Hz, 7H), 3.29-3.13 (m, 5H), 2.67-2.46 (m, 9H), 2.24 (s, 3H), 2.20-1.92 (m, 4H), 1.93-1.75 (m, 3H), 1.65 (dp, *J* = 16.0, 7.8 Hz, 2H), 1.43 (d, *J* = 38.9 Hz, 6H), 1.14-0.96 (m, 16H), 0.92 (t, *J* = 6.8 Hz, 6H). $C_{59}H_{90}N_{10}O_{14}S$ calcd. *m/z* = 1194.64 found [M+H]$^+$ 1195.51; [M+2H/2]$^+$ 599.09.

Example 3.168: (*S*,*E*)-*N*-(4-((14*R*,17*R*)-1-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)-5,6,7,8-tetrahydronaphthalen-1-ylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound AA).

Step 1: 2,2,2-trifluoro-*N*-(4-sulfamoyl-5,6,7,8-tetrahydronaphthalen-1-yl)acetamide (AA-1) synthesized from 5,6,7,8-tetrahydronaphthalen-1-amine according to General Procedure 8. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.04 (s, 1H), 7.79 (d, J = 8.4 Hz, 1H), 7.46 (s, 2H), 7.30 (d, J = 8.4 Hz, 1H), 3.14 (s, 1H), 2.77 (d, J = 15.4 Hz, 1H), 2.72-2.57 (m, 4H), 1.73 (p, J = 3.3 Hz, 4H).

Step 2: (*S*,*E*)-*N*-(4-amino-5,6,7,8-tetrahydronaphthalen-1-ylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (AA-2) synthesized from Boc-HTI-286-OH and Compound AA-1 using General Procedures 2, 3 and 7. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.74 (d, J = 8.7 Hz, 1H), 7.55 (d, J = 7.9 Hz, 2H), 7.48 (t, J = 7.6 Hz, 2H), 7.38 (t, J = 7.2 Hz, 1H), 6.60 (d, J = 8.7 Hz, 1H), 6.46 (d, J = 9.2 Hz, 1H), 5.00 (t, J = 10.0 Hz, 1H), 4.95-4.91 (m, 1H), 4.36 (s, 1H), 3.17 (s, 3H), 3.10-3.05 (m, 2H), 2.51 (s, 3H), 2.46 (t, J = 6.5 Hz, 2H), 2.10-2.02 (m, 1H), 1.88 (s, 3H), 1.87-1.75 (m, 4H), 1.47 (s, 3H), 1.38 (s, 3H), 1.07 (s, 9H), 0.92 (dd, J = 7.1 Hz, 6H). $C_{37}H_{55}N_5O_5S$ calcd. m/z = 681.39 found $[M+H]^+$ = 682.4.

Step 3: (*S*,*E*)-*N*-(4-((14*R*,17*R*)-1-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)-5,6,7,8-tetrahydronaphthalen-1-ylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide synthesized from Compound AA-2 and MT-NHS according to General Procedure 6. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.98 (d, J = 8.7 Hz, 1H), 7.62 (d, J = 8.7 Hz, 1H), 7.59-7.51 (m, 2H), 7.47 (dd, J = 8.5, 6.8 Hz, 2H), 7.42-7.30 (m, 1H), 6.83 (s, 2H), 6.50 (dd, J = 9.5, 1.8 Hz, 1H), 5.01 (t, J = 10.0 Hz, 1H), 4.93 (t, J = 4.1 Hz, 1H), 4.62 (td, J = 8.1, 7.5, 5.0 Hz, 1H), 4.37 (s, 1H), 4.29-4.18 (m, 1H), 3.75 (t, J = 6.0 Hz, 2H), 3.72-3.67 (m, 2H), 3.64 (td, J = 5.9, 1.5 Hz, 2H), 3.29-3.08 (m, 7H), 2.74 (d, J = 6.0 Hz, 2H), 2.62-2.46 (m, 5H), 2.20-1.94 (m, 4H), 1.91-1.75 (m, 7H), 1.70-1.58 (m, 2H), 1.48 (s, 3H), 1.38 (s, 3H), 1.07 (s, 9H), 1.00 (dd, J = 6.8, 3.4 Hz, 6H), 0.92 (t, J = 6.6 Hz, 6H). $C_{61}H_{92}N_{10}O_{14}S$ calcd. m/z = 1220.65 found $[M+H]^+$ 1221.48; $[(M+2H)/2]^+$ 611.39.

Example 3.169: (*S*,*E*)-*N*-(4-((14*R*,17*R*)-1-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)-3-fluorophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound BB).

Step 1: 2,2,2-trifluoro-*N*-(2-fluoro-4-sulfamoylphenyl)acetamide (BB-1) synthesized from 2-fluoroaniline according to General Procedure 8. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.58 (s, 1H), 7.85-7.66 (m, 3H), 7.56 (s, 2H).

Step 2: (*S*,*E*)-*N*-(4-amino-3-fluorophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (BB-2) synthesized from Boc-HTI-286-OH and Compound BB-1 using General Procedures 2, 3 and 7.

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.62-7.55 (m, 3H), 7.54 (s, 1H), 7.48 (t, J = 7.7 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 6.85 (t, J = 8.6 Hz, 1H), 6.45 (d, J = 9.3 Hz, 1H), 4.98 (t, J = 9.9 Hz, 1H), 4.92 (s, 1H), 4.34 (s, 1H), 3.16 (s, 3H), 2.50 (s, 3H), 2.12-2.00 (m, 1H), 1.88 (d, J = 1.4 Hz, 3H), 1.46 (s, 3H), 1.37 (s, 3H), 1.07 (s, 9H), 0.91 (dd, J = 6.8 Hz, 6H).

$C_{33}H_{48}FN_5O_5S$ calcd. m/z = 645.34 $[M+H]^+$ = 646.4

Step 3: (*S*,*E*)-*N*-(4-((14*R*,17*R*)-1-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)-3-fluorophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide synthesized from Compound BB-2 and MT-NHS according to General Procedure 6. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.42-8.28 (m, 1H), 7.91-7.77 (m, 2H), 7.58-7.51 (m, 2H), 7.47 (t, J = 7.8 Hz, 2H), 7.42-7.32 (m, 1H), 6.84 (s, 2H), 6.50 (dd, J = 9.3, 1.8 Hz, 1H), 5.02-4.90 (m, 2H), 4.67 (td, J = 7.9, 7.2, 4.8 Hz, 1H), 4.35 (s, 1H), 4.26 (t, J = 7.5 Hz, 1H), 3.76 (t, J = 6.1 Hz, 2H), 3.70 (td, J = 5.5, 1.2 Hz, 2H), 3.67-3.53 (m, 10H), 3.28-3.06 (m, 5H), 2.61-2.47 (m, 5H), 2.19-2.01 (m, 2H), 2.01-1.71 (m, 4H), 1.61 (dt, J = 15.2, 7.1 Hz, 2H), 1.46 (s, 3H), 1.36 (s, 3H), 1.13-0.95 (m, 16H), 0.91 (dd, J = 6.6, 4.9 Hz, 6H).

$C_{57}H_{85}FN_{10}O_{14}S$ calcd. m/z = 1184.60 found $[M+H]^+$ 1185.47; $[(M+2H)/2]^+$ 593.41.

Example 3.170: (*S*,*E*)-*N*-(4-((14*R*,17*R*)-1-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)-2-ethylphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound CC)

Step 1: *N*-(3-Ethyl-4-sulfamoylphenyl)-2,2,2-trifluoroacetamide (CC-1) synthesized from 3-ethylaniline according to General Procedure 8. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.48 (s, 1H), 7.89 (d, J = 8.5 Hz, 1H), 7.75-7.63 (m, 2H), 7.45 (s, 2H), 3.02 (q, J = 7.5 Hz, 2H), 1.24 (t, J = 7.4 Hz, 3H).

Step 2: (*S*,*E*)-*N*-(4-Amino-2-ethylphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (CC-2) synthesized from Boc-HTI-286-OH and Compound CC-1 using General Procedures 2, 3 and 7.

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.79 (d, J = 8.7 Hz, 1H), 7.55 (d, J = 7.9 Hz, 2H), 7.48 (t, J = 7.6 Hz, 2H), 7.37 (t, J = 7.4 Hz, 1H), 6.57 (d, J = 2.3 Hz, 1H), 6.54 (dd, J = 8.8, 2.4 Hz, 1H), 6.46 (d, J = 9.4 Hz, 1H), 5.01 (t, J = 10.0 Hz, 1H), 4.92 (s, 1H), 4.34 (s, 1H), 3.16 (s, 3H), 2.99-2.90 (m, 2H), 2.50 (s, 3H), 2.11-2.00 (m, 1H), 1.87 (d, J = 1.4 Hz, 3H), 1.47 (s, 3H), 1.38 (s, 3H), 1.22 (t, J = 7.5 Hz, 3H), 1.06 (s, 9H), 0.91 (dd, J = 6.6 Hz, 6H).

$C_{35}H_{53}N_5O_5S$ calcd. m/z = 655.38 $[M+H]^+$ = 656.4.

Step 3: (*S*,*E*)-*N*-(4-((14*R*,17*R*)-1-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)-2-ethylphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide synthesized from Compound CC-2 and MT-NHS according to General Procedure 6. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.04 (d, J = 8.8 Hz, 1H), 7.77 (d, J = 2.2 Hz, 1H), 7.67 (dd, J = 8.8, 2.2 Hz, 1H), 7.54 (d, J = 7.6 Hz, 2H), 7.46 (t, J = 7.7 Hz, 2H), 7.36 (t, J = 7.3 Hz, 1H), 6.83 (s, 2H), 6.51 (dd, J = 9.5, 1.9 Hz, 1H), 5.01 (t, J = 10.0 Hz, 1H), 4.92 (d, J = 8.4 Hz, 2H), 4.60-4.47 (m, 1H), 4.37 (s, 1H), 4.23 (d, J = 6.9 Hz, 1H), 3.82-3.72 (m, 2H), 3.69 (dd, J = 6.0, 4.5 Hz, 2H), 3.66-3.52 (m, 10H), 3.28-3.10 (m, 5H), 3.06 (q, J = 7.4 Hz, 2H), 2.58 (t, J = 6.0 Hz, 2H), 2.52 (s, 3H), 2.20-1.90 (m, 3H), 1.87 (s, 3H), 1.84-1.72 (m, 1H), 1.64-1.55 (m, 2H), 1.47 (s, 3H), 1.37 (s, 3H), 1.26 (t, J = 7.5 Hz, 3H), 1.10-0.96 (m, 15H), 0.91 (dd, J = 6.6, 4.0 Hz, 6H). $C_{59}H_{90}N_{10}O_{14}S$ calcd. m/z = 1194.64 found $[M+H]^+$ 1195.57; $[(M+2H)/2]^+$ 599.12

Example 3.171: (*S*,*E*)-*N*-(4-((14*R*,17*R*)-1-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)-3-ethylphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound DD).

Step 1: *N*-(2-ethyl-4-sulfamoylphenyl)-2,2,2-trifluoroacetamide (DD-1) synthesized from 2-ethylaniline according to general procedure 1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.21 (s, 1H), 7.80 (d, J = 2.1 Hz, 1H), 7.72 (dd, J = 8.2, 2.2 Hz, 1H), 7.48 (d, J = 8.3 Hz, 1H), 7.41 (s, 2H), 2.64 (q, J = 7.6 Hz, 2H), 1.16 (t, J = 7.5 Hz, 3H).

Step 2: (*S*,*E*)-*N*-(4-amino-3-ethylphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (DD-2) synthesized from Boc-HTI-286-OH and Compound DD-1 using General Procedures 2, 3 and 7. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.66 (d, J = 2.3 Hz, 1H), 7.61 (dd, J = 8.6, 2.3 Hz, 1H), 7.55 (d, J = 7.6 Hz, 2H), 7.48 (t, J = 7.7 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 6.71 (d, J = 8.5 Hz, 1H), 6.43 (dd, J = 9.3, 1.7 Hz, 1H), 4.96 (t, J = 9.9 Hz, 1H), 4.92 (s, 1H), 4.35 (s, 1H), 3.16 (s, 3H), 2.54 (dd, J = 7.4, 2.2 Hz, 2H), 2.51 (s, 3H), 2.12-1.99 (m, 1H), 1.87 (d, J = 1.4 Hz, 3H), 1.46 (s, 3H), 1.36 (s, 3H), 1.27 (t, J = 7.5 Hz, 3H), 1.07 (s, 9H), 0.91 (dd, J = 6.4 Hz, 6H). $C_{35}H_{53}N_5O_5S$ calcd. m/z = 655.38 $[M+H]^+$ = 656.5.

Step 3: (*S*,*E*)-*N*-(4-((14*R*,17*R*)-1-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)-3-ethylphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide synthesized from Compound DD-2 and MT-NHS according to General Procedure 6. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.97 (d, J = 2.3 Hz, 1H), 7.87 (dd, J = 8.5, 2.3 Hz, 1H), 7.77 (d, J = 8.5 Hz, 1H), 7.59-7.51 (m, 2H), 7.51-7.42 (m, 2H), 7.41-7.34 (m, 1H), 6.84 (s, 2H), 6.48 (dd, J = 9.4, 1.8 Hz, 1H), 4.98 (t, J = 9.9 Hz, 1H), 4.92 (d, J = 8.4 Hz, 1H), 4.64 (td, J = 8.4, 7.6, 3.7 Hz, 1H), 4.36 (s, 1H), 4.25 (d, J = 7.0 Hz, 1H), 3.82-3.67 (m, 4H), 3.67-3.53

(m, 10H), 3.29-3.09 (m, 5H), 2.77 (q, J = 7.5 Hz, 2H), 2.62-2.46 (m, 5H), 2.20-1.95 (m, 4H), 1.91-1.74 (m, 4H), 1.72-1.60 (m, 2H), 1.47 (s, 3H), 1.37 (s, 3H), 1.27 (t, J = 7.5 Hz, 3H), 1.12-0.95 (m, 16H), 0.91 (dd, J = 6.6, 4.6 Hz, 6H). $C_{59}H_{90}N_{10}O_{14}S$ calcd. m/z = 1194.64 found $[M+H]^+$ 1195.54; $[(M+2H)/2]^+$ 599.09.

Example 3.172: (*S*)-*N*-(4-(*N*-((*S*,*E*)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enoyl)sulfamoyl)phenyl)-1-((*S*)-1-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-methyl-12-oxo-3,6,9-trioxa-13-azapentadecane)pyrrolidine-2-carboxamide (Compound EE).

Synthesized from Compound N-1c and Boc-Ala-Pro-OH according to General Procedure 10, followed by Boc-removal according to General Procedure 7 and MT-NHS installation according to General Procedure 6 prior to purification by preparative HPLC. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.99 (d, J = 8.9 Hz, 2H), 7.81 (d, J = 8.5 Hz, 2H), 7.55 (d, J = 7.5 Hz, 2H), 7.48 (t, J = 7.7 Hz, 2H), 7.38 (t, J = 7.3 Hz, 1H), 6.84 (s, 2H), 6.54-6.42 (m, 1H), 5.07-4.95 (m, 2H), 4.67 (t, J = 6.8 Hz, 1H), 4.57 (dd, J = 8.4, 4.6 Hz, 1H), 4.35 (s, 1H), 3.95-3.83 (m, 1H), 3.80-3.66 (m, 5H), 3.61 (dd, J = 18.6, 4.6 Hz, 10H), 3.16 (s, 3H), 2.58-2.42 (m, 5H), 2.36 (d, J = 18.0 Hz, 1H), 2.23-1.98 (m, 4H), 1.86 (d, J = 1.4 Hz, 3H), 1.46 (s, 3H), 1.43-1.31 (m, 6H), 1.07 (s, 10H), 0.91 (t, J = 6.3 Hz, 6H). $C_{59}H_{90}N_{10}O_{14}S$ calcd. m/z = 1078.54 found $[M+H]^+$ 1079.48; $[(M+2H)/2]^+$ 540.27.

Example 3.173: (*S*,*E*)-*N*-(4-((*S*)-6-Amino-2-((*S*)-2-(3-(2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethoxy)propanamido)-3-phenylpropanamido)hexanamido)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound FF).

The title compound was prepared from Compound N-1c and Fmoc-Phe-Lys(Boc)-OH according to General Procedure 10, followed by Fmoc removal according to General Procedure 5, acylation with MT-NHS according to General Procedure 6 and deprotection according to General Procedure 7 prior to purification by preparative HPLC. $C_{61}H_{87}N_9O_{13}S$ calcd. m/z = 1185.6 found $[M+H^+]^+$ = 1186.6 and $[(M+2H^+)/2]^{2+}$ = 593.9.

Example 3.174: (*S*,*E*)-*N*-((4-((14*S*,17*S*)-17-(4-Aminobutyl)-1-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-3,6,9-trioxa-13,16-diazaoctadecanamido)phenyl)sulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound GG).

The title compound was prepared from Compound N-1c and Fmoc-Val-Lys(Boc)-OH according to General Procedure 10, followed by Fmoc removal according to General Procedure 5, acylation with MT-NHS according to General Procedure 6 and deprotection according to General

Procedure 7 prior to purification by preparative HPLC. $C_{57}H_{87}N_9O_{13}S$ calcd. *m/z* = 1137.6 found $[M+H^+]^+$ = 1138.5 and $[(M+2H^+)/2]^{2+}$ = 569.8.

Example 3.175: (*S*,*E*)-*N*-(4-((2*S*,5*S*,8*R*)-2-(4-Aminobutyl)-5-benzyl-15-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-8-methyl-4,7,10-trioxo-13-oxa-3,6,9-triazapentadecanamido)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound HH).

The title compound was prepared from Compound N-1c and Fmoc-Ala-Phe(D)-Lys(Boc)-OH according to General Procedure 10. The resulting material, purified by flash chromatography was then subject to General Procedure 5 to remove the Fmoc protecting group, followed by treatment with MT-NHS according to General Procedure 6 and deprotection according to General Procedure 7 prior to purification by preparative HPLC. $C_{64}H_{92}N_{10}O_{14}S$ calcd. *m/z* = 1256.7 found $[M+H^+]^+$ = 1258.3 and $[(M+2H^+)/2]^{2+}$ = 630.2.

Example 3.176: (*S*,*E*)-*N*-(4-((2*S*,5*S*,8*R*)-2-(4-Aminobutyl)-5,8-dibenzyl-15-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-4,7,10-trioxo-13-oxa-3,6,9-triazapentadecanamido)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound II).

The title compound was prepared from Compound N-1c and Fmoc-Phe-Phe(D)-Lys(Boc)-OH according to General Procedure 10, Fmoc-removal via General Procedure 5, reaction with MT-NHS according to General Procedure 6 and deprotection according to General Procedure 7, followed by prep HPLC purification. $C_{69}H_{94}N_{10}O_{14}S$ calcd. *m/z* = 1332.7 found $[M+H^+]^+$ = 1334.3 and $[(M+2H^+)/2]^{2+}$ = 668.2.

Example 3.177: (*S*,*E*)-*N*-(2-((14*S*,17*S*)-1-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound JJ).

Step 1: 2,2,2-Trifluoro-*N*-(2-sulfamoylphenyl)acetamide (JJ-1) was made from 2-aminobenzenesulfonamide according to General Procedure 1.

Step 2: (*S*,*E*)-*N*-(2-Aminophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (JJ-2) was made from Compound JJ-1 and Boc-HTI-286-OH according to General Procedures 2 and 3. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.75 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.55 (d, *J* = 7.8 Hz, 2H), 7.48 (t, *J* = 7.7 Hz, 2H), 7.38 (t, *J* = 7.4 Hz, 1H), 7.33-7.27 (m, 1H), 6.81 (d, *J* = 8.2 Hz, 1H), 6.69 (t, *J* = 7.5 Hz, 1H), 6.49 (dd, *J* = 9.1, 1.5 Hz, 1H), 4.97 (t, *J* = 10.1 Hz, 1H), 4.92 (s, 1H), 4.35 (s, 1H), 3.17 (s, 3H), 2.51 (s, 3H), 2.07

(m, 1H), 1.88 (d, J = 1.4 Hz, 3H), 1.46 (s, 3H), 1.36 (s, 3H), 1.06 (s, 9H), 0.92 (t, J = 6.8 Hz, 6H).$C_{33}H_{49}N_5O_5S$ calcd. m/z = 627.35 amu; found $[M+H]^+$ = 628.36, $[M+Na]^+$ = 650.37, $[(M+2H)/2]^{2+}$ = 314.76.

Step 3: *tert*-Butyl ((*S*)-1-(((*S*)-1-((2-(*N*-((*S*,*E*)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-((*tert*-butoxycarbonyl)methylamino)-3-phenylbutanamido)butanamido)hex-2-enoyl)sulfamoyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (JJ-3) was generated from Compound JJ-2 and Boc-Val-Cit-OH according to General Procedure 10. $C_{54}H_{85}N_9O_{12}S$ calcd. m/z = 1083.60 amu; found $[M+H]^+$ = 1084.8, $[M+Na]^+$ = 1106.7.

Step 4: (*S*,*E*)-*N*-(2-((*S*)-2-((*S*)-2-Amino-3-methylbutanamido)-5-ureidopentanamido)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (JJ-4) was generated from Compound JJ-3 according to General Procedure 7. $C_{44}H_{69}N_9O_8S$ calcd. m/z = 883.50 amu; found $[M+H]^+$ = 884.6, $[M+Na]^+$ = 906.6, $[(M+2H)/2]^{2+}$ = 442.8.

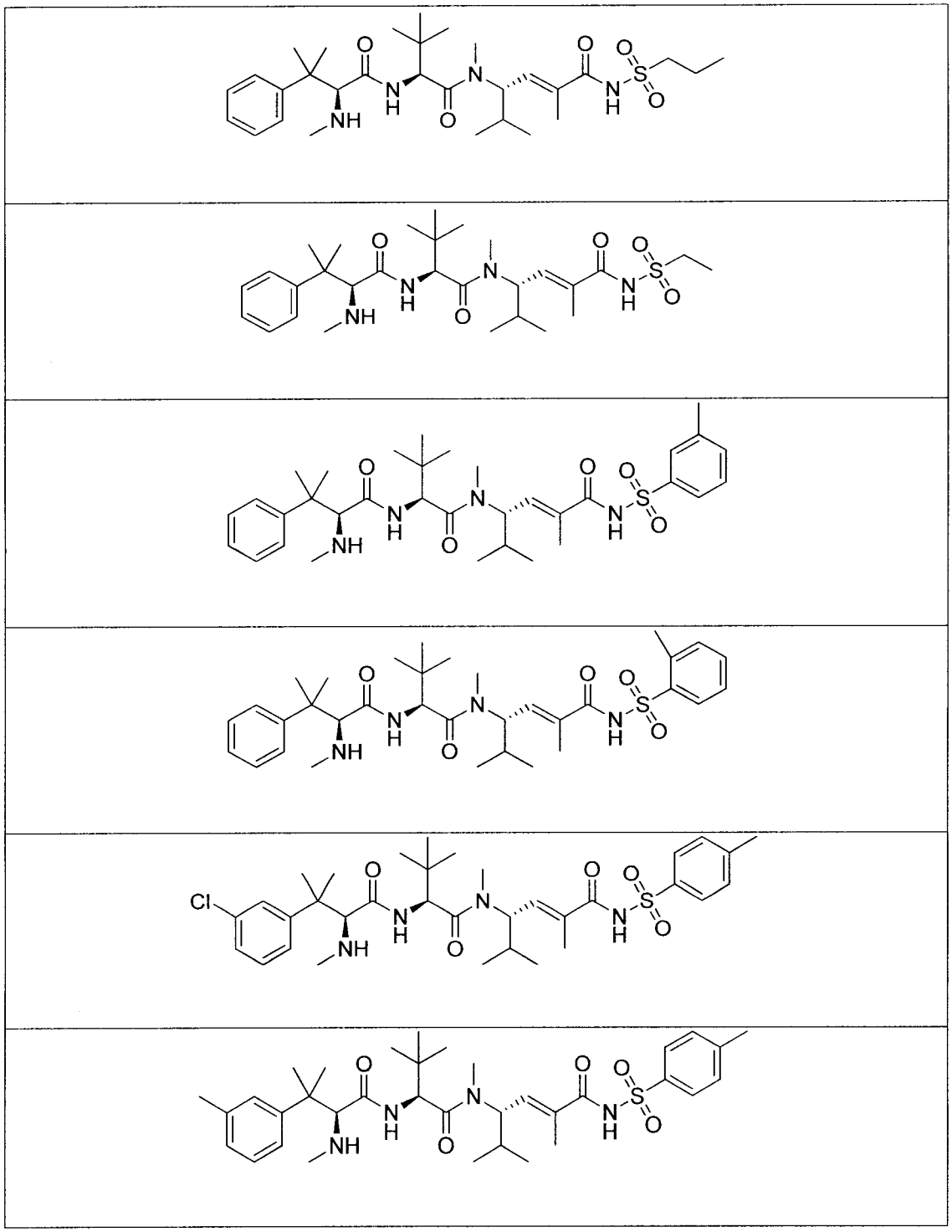
Step 5: (*S*,*E*)-*N*-(2-((14*S*,17*S*)-1-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecanamido)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide was generated from Compound JJ-4 and MT-NHS according to General Procedure 6 before purification by preparative HPLC-MS. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.16 (d, J = 8.3 Hz, 1H), 7.95 (dd, J = 8.0, 1.6 Hz, 1H), 7.50 (d, J = 7.9 Hz, 2H), 7.42 (dt, J = 15.5, 7.8 Hz, 3H), 7.29 (t, J = 7.3 Hz, 1H), 7.19 (t, J = 7.5 Hz, 1H), 6.85 (s, 2H), 6.62 (d, J = 9.3 Hz, 1H), 4.66 (s, 1H), 4.61 (dd, J = 9.1, 4.5 Hz, 1H), 4.37 (d, J = 6.9 Hz, 1H), 3.76 (dd, J = 7.5, 5.7 Hz, 2H), 3.73-3.67 (m, 2H), 3.67-3.56 (m, 10H), 3.29-3.13 (m, 4H), 3.11 (s, 3H), 2.70 (s, 6H), 2.65-2.49 (m, 2H), 2.22 (s, 3H), 2.11 (d, J = 7.5 Hz, 2H), 2.00 (dt, J = 17.2, 6.2 Hz, 2H), 1.86 (d, J = 1.4 Hz, 3H), 1.66 (dt, J = 14.5, 7.8 Hz, 2H), 1.01 (d, J = 13.3 Hz, 15H), 0.87 (dd, J = 21.4, 6.6 Hz, 6H). $C_{57}H_{86}N_{10}O_{14}S$ calcd. m/z = 1166.60 amu; found $[M+H]^+$ = 1167.8, $[M+Na]^+$ = 1189.9, $[(M+2H)/2]^{2+}$ = 584.4.

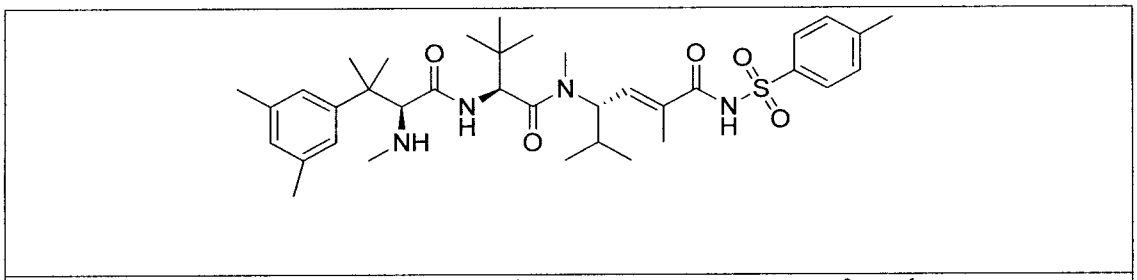
Example 3.178: MT-Val-Cit-OH: (14*R*,17*R*)-1-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-14-isopropyl-12,15-dioxo-17-(3-ureidopropyl)-3,6,9-trioxa-13,16-diazaoctadecan-18-oic Acid.

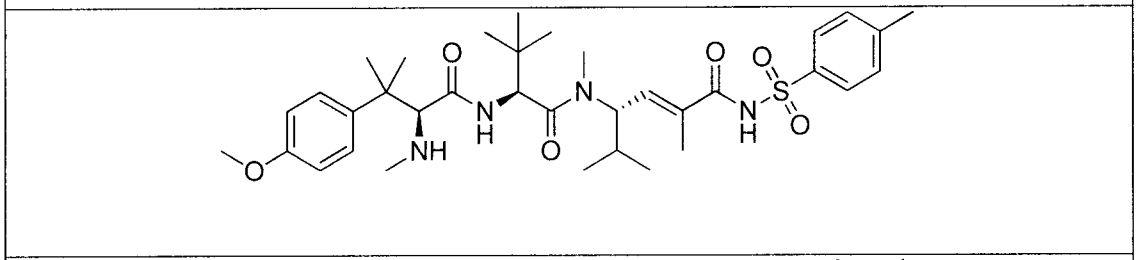
The title compound was prepared from H-VC-OH (0.50g, 1.287 mmol)) and MT-NHS (0.512g, 1.287 mmol) with *N*,*N*-di-isopropylethylamine (0.448 mL, 2 equiv) in dioxanes (0.50mL). Upon consumption of the starting material (~16h, evaluated by HPLC-MS), the reaction was concentrated *in vacuo* and the resulting oil was purified by preparative HPLC-MS. Lyophilization of the desired fractions afforded the title compound as a white powder (0.351 g, 63%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.76 (s, 2H), 4.54-4.59 (m, 1H), 4.33 - 4.38 (m, J = 7.6 Hz, 1H), 3.85 – 3.70 (m, 5H), 3.60-3.68 (m, 10H), 3.18-3.22 (m, 2H), 2.55-2.62 (m, 2H), 2.10-2.18 (m, 1H), 1.90-2.05 (m, 1H), 1.72-1.85 (m, 1H), 1.54-1.65 (m, 2H), 0.98 (t, J = 6.6 Hz, 6H).

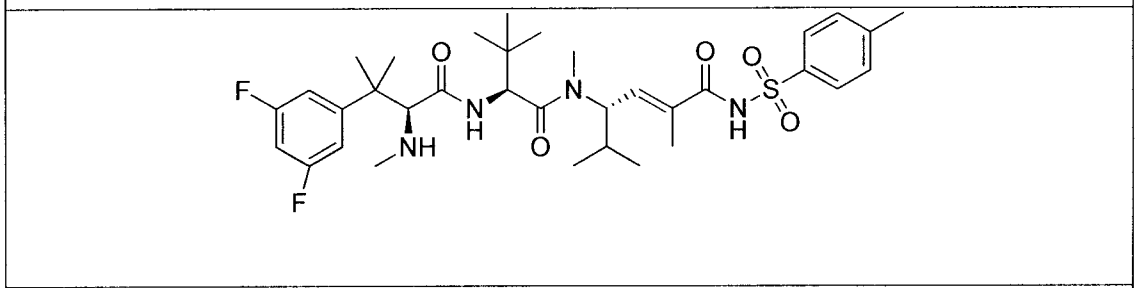
Example 3.179: Other Representative Compounds (P^1).

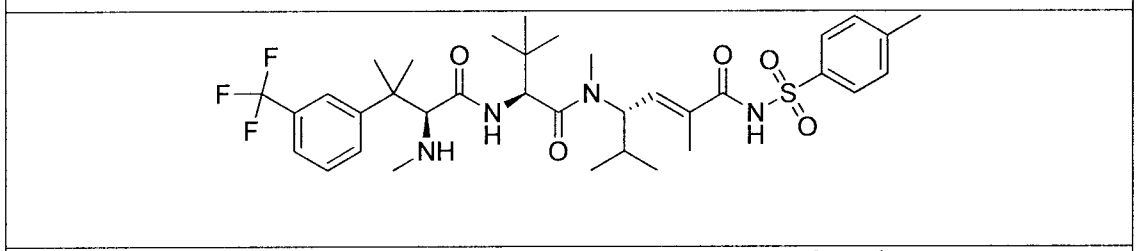
The following representative compounds may be prepared according to the foregoing procedures. As recognized by the artisan of reasonable skill, the following compounds are synthetically accessible using the disclosure of WO 2004/026293 to achieve the precursor reactant and applying General Procedures with the appropriate sulfonamide.

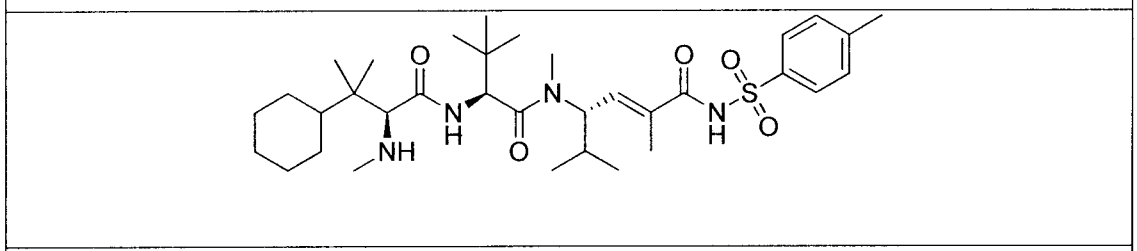


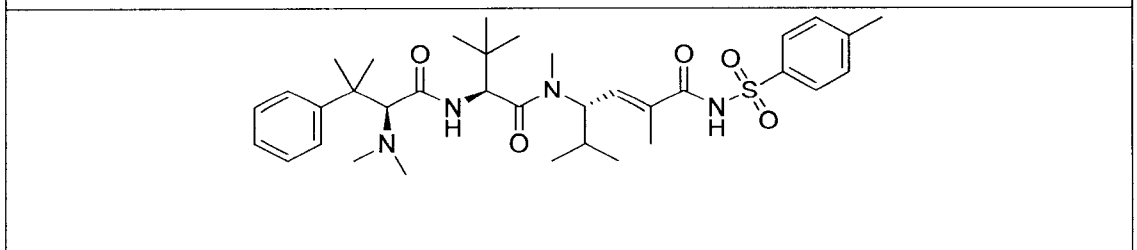


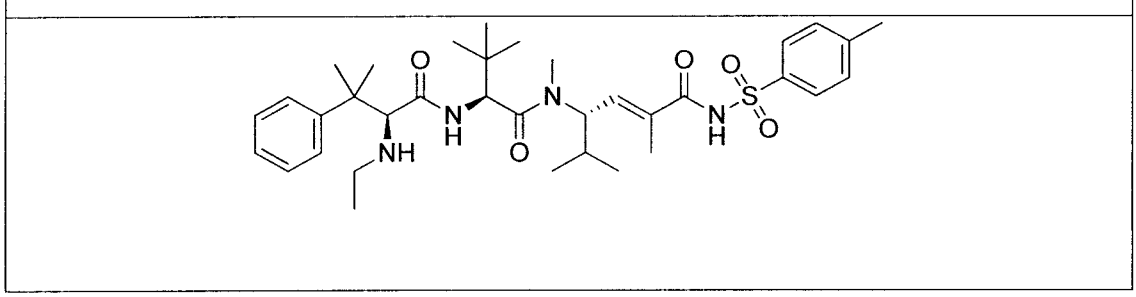


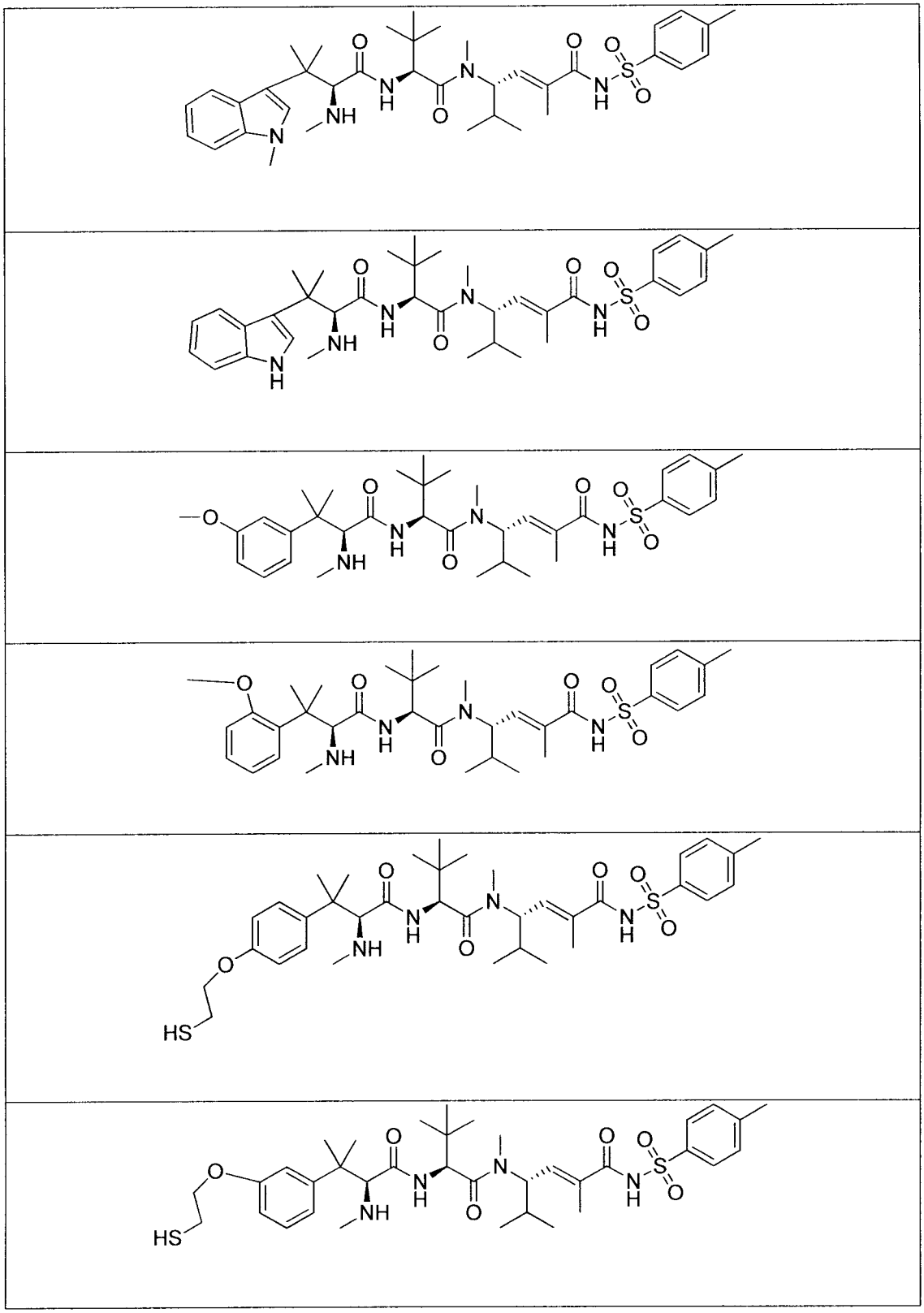


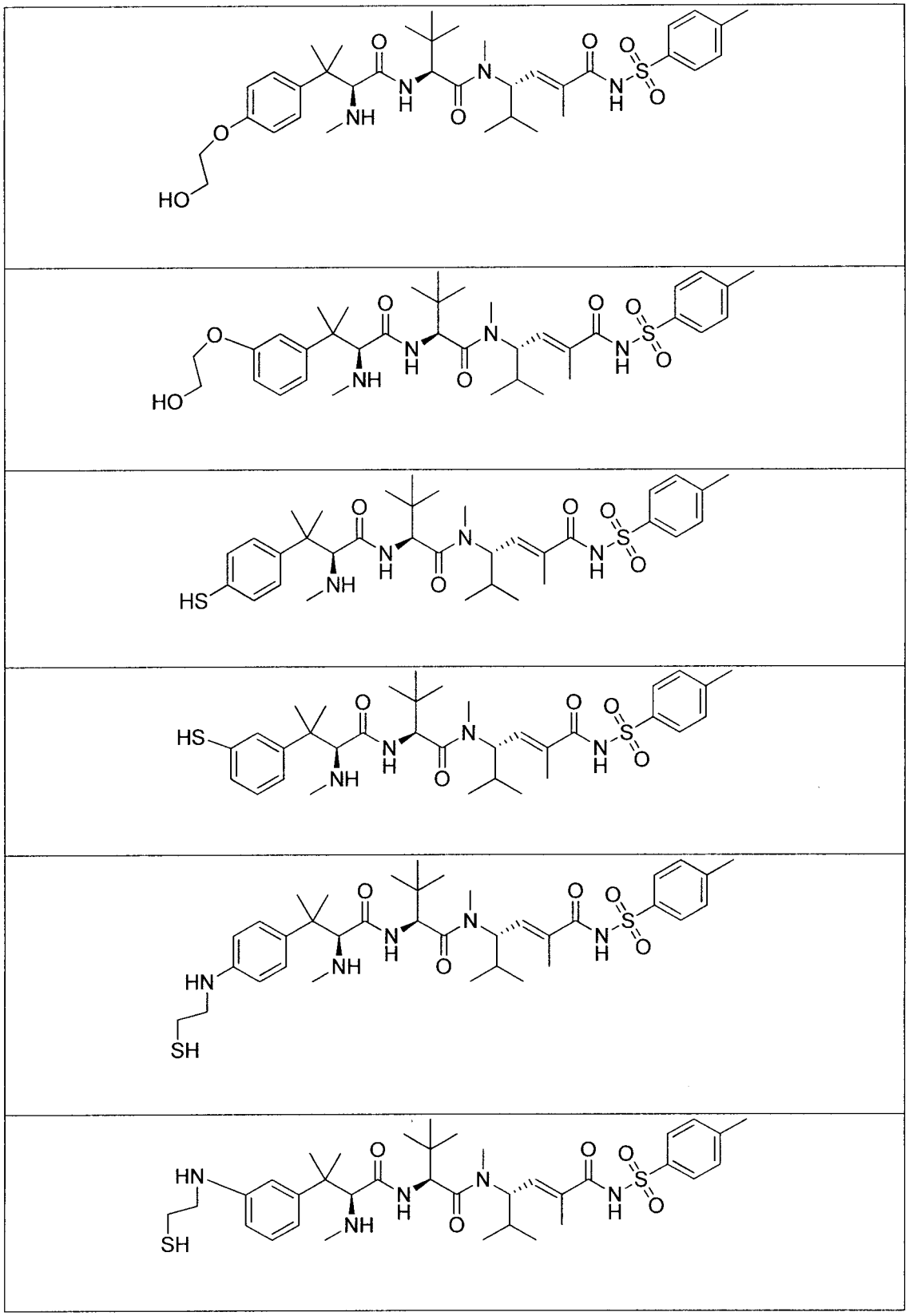


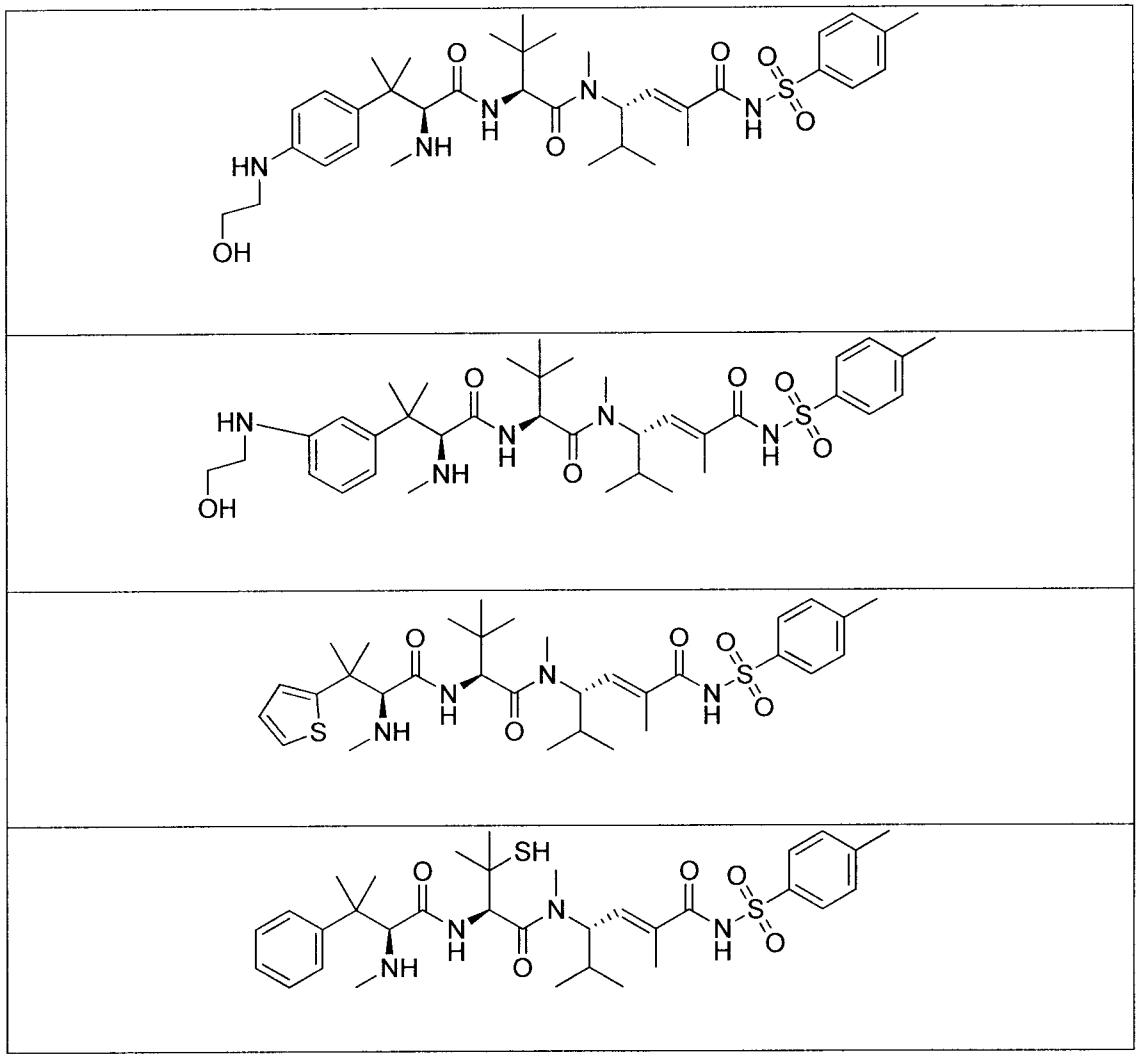












Example 4: Preparation of Certain Compounds of Formula I.

Proteins:

Truncated recombinant VAR2CSA proteins (see Dahlbäck *et al.* for domain boundaries – J. Biol. Chem. 286: 15908-15917) were expressed in *E. coli* or eukaryotic expression systems and purified according to the general methods below.

General purification methods for recombinant VAR2CSA proteins

Recombinant DBL1-ID2a and ID1-ID2a were produced in stable transfected Drosophila Schneider-2 (S2) cells or in baculovirus transfected insect cells. Harvested culture supernatants were diafiltrated using an Äcta Crossflow. HIS tagged versions of the two proteins were purified on Ni++ 5 mL HisTrap HP columns and eluted with 350 mM imidazole. The eluted fractions were further purified on a Superdex200 GF column (in : 1xPBS, 0.5M NaCl, pH 7.2 with 1 CMPIT protease inhibitor tablet per 300 mL buffer) and monomeric fractions were selected for further analyses and toxin coupling. The VAR2CSA recombinant proteins with or without poly histidine tag were also purified using standard ion exchange columns (negative and positive selection) and further purified on columns utilizing a hydrophobic interaction as well as ionic interaction. The proteins were also purified on columns with bound nanobody or antibody specific to VAR2CSA relying on a specific

interaction between the VAR2CSA specific antibody reagents and the VAR2CSA protein. The proteins were eluted in a NaCl gradient or a pH gradient going from 7.4 down to 2, followed by immediate neutralization in a basic buffer. To stabilize protein during purification we used (each alone or in combination) the following compounds: Hydroxyectoine, Sucrose, EDTA, D-Sorbitol, Xylitol, D-(+)-Trehalose dehydrate, Betaine monohydrate, Tryptone, Gly-gly-gly ,Gly-gly, 6-Aminohexanoic acid, L-Serine, β-Alanine, L-Histidine, Glycine, L-Arginine, L-Arginine+L-Glutamic acid, Taurine, Non Detergent Sulfobetaine 211 (NDSB-211)

Recombinant VAR2CSA proteins expressed in C3029H or C3030H *E. coli* cells were purified from cell lysates produced by sonication. Polyhistidine tagged proteins were purified on Ni++ 5 mL HisTrap HP columns and eluted with 350 mM imidazole. The eluted fractions were further purified on a Superdex200 GF column (in : 1xPBS, 0.5M NaCl, pH 7.2 with 1 CMPIT protease inhibitor tablet per 300 mL buffer) and monomeric fractions were selected for further analyses and toxin coupling.

Cell Binding Panel:

Numerous cancer cell lines were screened for binding to VAR2CSA by FACS. Table 6 summarizes the binding of recombinant DBL1-ID2a to a panel of cancer cell lines. Signal from VAR2CSA staining (mean fluorescence intensity of bound V5-tagged VAR2CSA detected by mIgG2ak anti-V5-FITC) is compared to background (mean fluorescence intensity of bound V5-tagged VAR2CSA detected by mIgG2a-FITC isotype control antibody).

General Method: Binding of Recombinant VAR2CSA to Cancer Cells by FACS

Cells were established in logarithmic growth in their respective growth medium prior to the assay. On the day staining was performed, culture medium was aspirated and discarded. 5 mL of PBS was added to the culture vessel to rinse cells and PBS was then removed by aspiration. At this point cell dissociation buffer (3 mL; Sigma C5914) was added to adherent cell lines and the cells were incubated until detachment was observed under a microscope. The cell dissociation reagent was neutralized with 7 mL of serum containing culture medium and cell viability was determined using Trypan Blue exclusion assay. Alternatively, suspension cells were assayed for viability directly after washing with PBS. Cells were added to the bottom of 96 well V-bottomed plates (50,000 cells/well) and pelleted by centrifugation (400 × g, 3 min). After removal of supernatant, 15 µL of V5-tagged recombinant VAR2CSA (400 nM) or FACS buffer (PBS + 1% FBS) was added to resuspend the cell pellet. After incubation on ice for a period of one hour, plates were washed by addition of 200 µL FACS buffer, centrifugation (400 × g, 3 min), removal of supernatant, resuspension of the cell pellet in 200 µL of FACS buffer with disruption of the pellet, centrifugation (400 × g, 3 min), and

finally removal of supernatant. Cell pellets were resuspended in 25 µL of either mIgG2ak anti-V5-FITC (1:100 dilution), or mIgG2a-FITC (2 µg/mL dilution) and 7A.A.D (2.5 µg/mL). Plates were incubated on ice for 0.5 hours before washing cells as described above. The cell pellets were then resuspended in 75 µL FACS buffer and analyzed by flow cytometry. Data are represented as live cell (7-AAD negative population) geometric mean fluorescence in the FITC channel

Table 6. Cell line binding by recombinant VAR2CSA as assessed by FACS.

	VAR2CSA Staining		Background Controls
Cell Line	**MFI**	**Signal:Background Ratio**	**MFI (Secondary alone/Isotype)**
Ramos	4044	7	584
A-172	52938	30	1766
NCI-H358	45991	26	1800
HT-29	16405	9	1845
HCT-15	6470	8	800
A549	81032	52	1561
MDA-MB-231	49745	43	1149
HCC-1954	61444	18	3353
OE19	51471	9	5565
OVCAR-3	32114	11	2796
OV-90	20503	6	3644
BxPC-3	33962	7	4778
MIA PaCa-2	9031	4	2277
HPAF-II	11745	3	3540
PANC-1	18348	5	3586
AsPc-1	30015	11	2856
CCRF-CEM	6395	17	367
AML-193	2943	4	741
Jurkat	9363	24	389
PC-3	32925	19	1775
DU145	21069	11	1854
RT112/84	38410	21	1820
CaOV3	19852	5	3724
SKOV3	61831	29	2144

	VAR2CSA Staining		Background Controls
Cell Line	**MFI**	**Signal:Background Ratio**	**MFI (Secondary alone/Isotype)**
MCF7	18259	10	1879
A-431	54090	21	2592
NCI-N87	4487	5	995
Colo-205	449061	233	1926
T47-D	83146	52	1595
MDA-MB-231	82323	53	1566
MDA-MB-468	70162	31	2287
Colo-205	108367	73	1479
253JB-V	32875	24	1398
Myla 2059	89669	94	958
K562 wt	20211	15	1315
K562 #16	2018	1	1773
K562 #14	2215	2	1386
PC-3	46023	29	1603
UM-UC3	73627	47	1553
A549	109308	33	3312
MG-63	75389	28	2729
T47-D	74285	59	1257
HUVEC	9703	5	2024
MDA-MB-231	27663	14	1950
MDA-MB-231	91666	46	1986
U2OS	96707	55	1761
RH-30	43355	11	4058

Exemplary Conjugation Conditions:

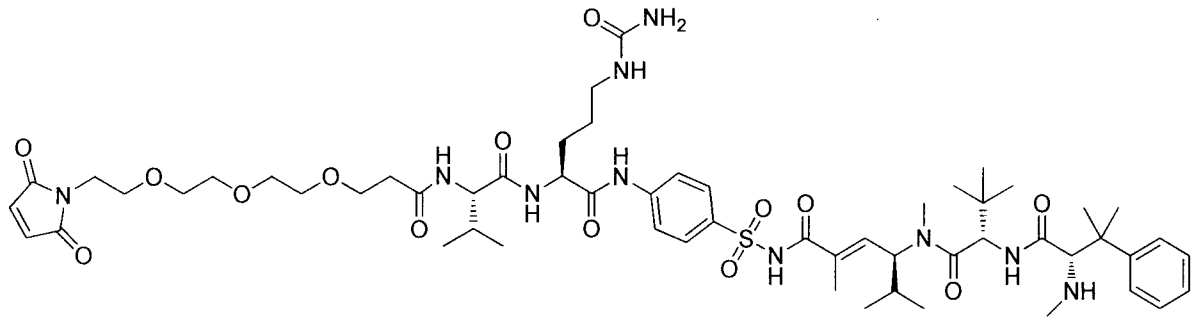
General Method: Coupling at cysteine residues with maleimide functionalized toxins

An aliquot of truncated recombinant VAR2CSA protein DBL1-ID2a (lot MP1255; 197 µL; 181 µg) was thawed on ice and handled on ice thereafter. To the protein solution was added maleimide functionalized toxin (2.4 µL of a 10 mM DMSO stock solution; 15.0 equivalents to protein) with thorough and immediate mixing. The reaction was allowed to proceed for a period of 90 minutes after which time the solution was applied to a Zeba spin

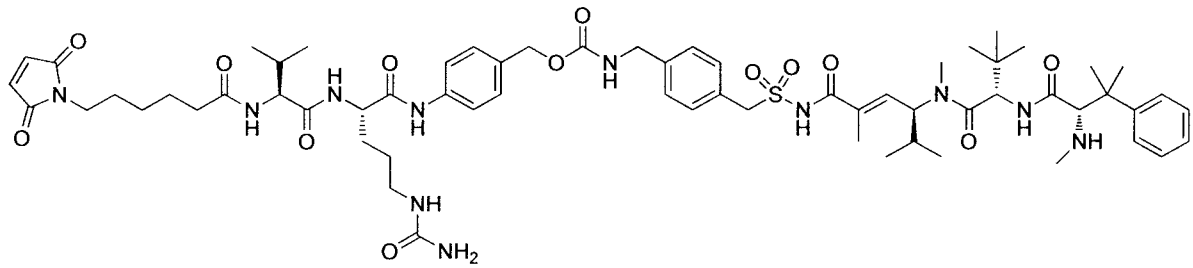
desalting column (Pierce, product #87766, lot #198863) preconditioned with PBS. The recovered eluate was aliquoted and frozen at -80 °C prior to use.

Examples of maleimide functionalized toxins

Compound O



MCvcPABC-3.90

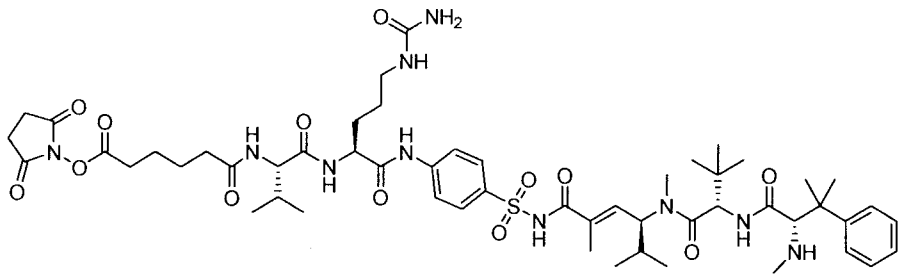


General Method: Coupling at lysine residues with NHS-ester functionalized toxins

An aliquot of truncated recombinant VAR2CSA protein DBL1-ID2a (197 µL; 181 µg) was thawed on ice and handled on ice thereafter. To the protein solution was added an NHS ester functionalized toxin (3.2 µL of a 5 mM DMSO stock solution; 10.0 equivalents to protein) with thorough and immediate mixing. The reaction was allowed to proceed for a period of 90 minutes after which time the solution was applied to a Zeba spin desalting column (Pierce, product #87766, lot #198863) preconditioned with PBS. The recovered eluate was aliquoted and frozen at -80 °C prior to use.

Example of N-hydroxysuccinimidyl ester functionalized toxins

Compound KK



Standard Characterization Procedures:

1. Purified drug conjugates were assessed for total protein content (BCA assay, Pierce microBCA protocol).

2. VAR2CSA conjugates were evaluated alongside unmodified VAR2CSA for degradation and or oligomerization via SDS-PAGE under both reducing and non-reducing conditions, using Coomassie Blue staining and appropriate protein standards.

3. Purified VAR2CSA drug conjugates were evaluated (alongside unmodified VAR2CSA) to assess drug loading by Size Exclusion Ultra High Performance Liquid Chromatography-Mass Spectrometry (SEC-UPLC-QTof-MS). An average drug to VAR2CSA ratio (DVR) was estimated from the deconvolved mass spectrum via evaluation of peak intensities corresponding to VAR2CSA + 1 drug, 2 drugs, 3 drugs, etc.

General Method: SEC-UPLC-ESI-ToF-MS Analysis of VAR2CSA Conjugates for DVR Determination

Frozen aliquots of VAR2CSA conjugates were thawed prior to SEC-EsiTofMS analysis. The SEC analysis was performed with a WATERS Acquity UPLC BEH200 SEC 1.7 μm 4.6 x 150 mm column and mobile phase composed of Acetonitrile/Water/Trifluoroacetic acid/Formic acid, (30/70/0.1/0.1, v/v/v/v% - trifluoroacetic acid was omitted in some instances) , and a column temperature of 30 °C. 10 μL of sample was injected onto the SEC column. The elution time was 11.0 min. VAR2CSA and the VAR2CSA conjugates eluted at ~ 3.4 min. The MS total ion current (TIC) data was acquired on a Quatro-Premier QToF mass spectrometer with an electrospray ion source (WATERS 20 Corporation) over 500- 4500 *m/z* range using MassLynx data acquisition software (Waters Corporation). Sample component mass data was acquired in the positive ion V-mode , and the Esi source was operated at a source temperature: 150 °C, desolvation temperature: 350 °C, desolvation gas: 800 L/hr, Sample cone voltage: 60 V, Capillary voltage: 3.0 kV, desolvation gas: Nitrogen, and collision gas: Argon. The summed TIC mass spectra of the sample peak were deconvoluted by the MaxEnt1 algorithm to generate the neutral mass data of the sample component.

Exemplary Data for Average Drug to VAR2CSA Ratio Determination:

VAR2CSA (DBL1-ID2a) conjugates prepared by modification of cysteine and lysine residues with maleimide functionalized toxin (Compound O) or NHS functionalized toxin (Compound KK) were evaluated for toxin loading by SEC-MS as described in the General Method. The estimated mass obtained for the unmodified precursor protein was 114162 Da.

Figure 8 shows the SEC-UPLC-QTof-MS MaxEnt1 processed intact mass of VAR2-Compound O. The MS signals at 115323 Da, 117662 Da and 119999 Da are consistent with conjugation of 1, 3 and 5 toxins, with a mean conjugation level of ~4 toxins per protein.

Figure 9 shows the: SEC-UPLC-QTof-MS MaxEnt1 processed intact mass of VAR2-Compound KK. The profile of the deconvolved MS data is consistent with conjugation of up to 5 toxins (Compound KK), but with a mean drug load of ~2.5 drugs.

4. Conjugates were evaluated for the presence of unconjugated linker-toxin or free toxin by UPLC-ESI-Triple quadrupole mass spectrometry. Any unconjugated-drug in the purified product was extracted by protein precipitation with 0.1% formic acid in acetonitrile. The extract was filtered through a 3 KDa molecular weight cutoff filter (Amicon). The resulting filtrate was analyzed by RP-UPLC-MSMS to quantify the amount of unconjugated-drug. The amount of unconjugated- drug was quantified against a free drug (and free drug-linker) calibration curve. The UPLC-MSMS equipment used consists of an Ultra Performance Liquid Chromatograph (Waters Acquity™ UPLC) with tandem Waters Acquity™ PDA and TQD detectors. Control of the equipment and data acquisition were performed by PC computer and Waters Empower-2 ™ chromatography software.

Detection of Compound O in VAR2-Compound O sample prepared by general conjugation method, above.

Exemplary data for free ESI-MS based free drug analysis is shown in Figure 10. A similar result was obtained for free drug Compound 886 alone.

5. VAR2CSA drug conjugates were assessed by flow cytometry +/- CSA (Sigma C9819) for binding to "benchmark" Myla 2059 cutaneous T cell lymphoma cell line in order to estimate the specificity of binding. The binding of tumor-cell plCSA can be inhibited by incubating the VAR2CSA with excess soluble CSA. This sequesters VAR2CSA in solution and inhibits plCSA-binding on the cell-surface. It is assumed that cell surface staining observed in the presence of excess soluble CSA would indicate non-specific binding of the VAR2CSA to the cells.

General Method: Flow Cytometry Based Determination of VAR2CSA-Drug Conjugate Binding Specificity

Cells were established in logarithmic growth in their respective growth medium prior to the assay. On the seeding day, cells were aspirated, resuspended in PBS+2%FBS, counted and cell viability was determined using Trypan Blue exclusion assay. Cells were added to the bottom of 96 well V-bottomed plates (50,000 cells/well) and pelleted by centrifugation (400 × g, 3 min). After removal of supernatant, 62.5 µL of VAR2CSA or VAR2CSA conjugates (+/- 400 µg/mL CSA) or FACS buffer were added at one or more concentrations (400 nM to obtain saturated binding; titration down to ~ 1 nM) and the cell pellets were resuspended.

After incubation on ice for a period of 0.5 hours, plates were washed by addition of 200 µL FACS buffer, centrifugation (400 × g, 3 min), removal of supernatant, resuspension of the cell pellet in 200 µL of FACS buffer with disruption of the pellet, centrifugation (400 × g, 3 min), and finally removal of supernatant. Cell pellets were resuspended in 25 µL of either mIgG2ak anti-V5-FITC (1:100 dilution), or mIgG2a-FITC (2 µg/mL dilution) and 7A.A.D (2.5 µg/mL). Plates were incubated on ice for 0.5 hours before washing cells as described above. The cell pellets were then resuspended in 50 µL FACS buffer and analyzed by flow cytometry. Data are represented as live cell (7-AAD negative population) geometric mean fluorescence in the FITC channel.

Figures 11-13 show the specificity of certain compounds of Formula I binding to the Myla2059 cell line.

6. VAR2CSA drug conjugates were evaluated for in vitro potency against a broad array of cancer cell lines. Cells were incubated with variable concentrations of each conjugate, incubated under growth conditions and assessed for cell viability at a predetermined time point. Table 7 summarizes the cytotoxic activity of a representative drug conjugate against 34 human cancer cell lines. As a negative control, CSA "knockout" cell lines K562 #14 (or K562 #16) could also be treated with VAR2CSA drug conjugates. K562 #14 and #16 showed low binding to VAR2CSA by FACS, thus suggesting that significant cytotoxic effect of the conjugates on this cell line would arise only in the presence of excess free toxin.

General Method: Cellular Cytotoxicity Assay of VAR2CSA-Drug Conjugates

On the day prior to adding test articles, adherent cells were added to opaque-walled 96-well tissue culture-treated microtiter plates using complete growth medium at a density of 2500 cells/100 microliter (µL) of medium (Colo205 cells were added at a density of 5000 cells/100 µL of medium). The cells were incubated for one night at 37 °C/5% CO_2 to allow the cells to attach to the microtiter plate surface. On the day that test articles were added, suspension cell lines were added to separate 96-well microtiter plates at 2500 cells/100 µL using the recommended growth medium. Drug conjugates were diluted directly in growth medium at five-times the desired final concentration and were then titrated 1:3, eight steps. A control with no test article present (growth medium alone) was included on each microtiter plate in sextuplicate. The prepared compound/protein-drug conjugate titrations were added (twenty-five µL/well) in triplicate to cells. The cells and titrations were incubated at 37 °C/5% CO_2 for five nights. After the incubation, cell viability is measured using CellTiter-Glo® reagent by adding thirty µL of prepared CellTiter-Glo® to each assay well. The assay is incubated for at least twenty minutes in the dark prior to measuring emitted luminescence using a microplate luminometer (500 ms integration time). The collected relative luminescence units (RLU) are converted to %

cytotoxicity using the Growth medium alone control mentioned above (% Cytotoxicity = 1 - [Well RLU/average medium alone control RLU]).

Exemplary cytotoxicity data is shown in Figures 14-19.

Table 7. Collected Cytotoxicity Data for DBL1-ID2a-toxin conjugates on a Panel of Human Cancer Cell Lines. Average Loading of ~4 toxins per DBL1-ID2a molecule.

		EC_{50} (nM)	
		Toxin	
Cell Line	**Cancer Tissue Type**	**Compound O**	**MCvcPABC-3.90**
BxPC-3	Pancreas	5.8	
NCI-N87	Stomach	6.8	
HCC1954	Breast	1.7	
Capan-2	Pancreas	~100	
AsPC-1	Pancreas	5.2	
Jurkat	T cell leukemia	~11	
MiaPaCa	Pancreas	30.9	
OVCAR-3	Ovary	1.7	
Karpas 299	T Cell Lymphoma	1	
H1975	Non-small cell Lung	1.7	
NCI-H358	Non-small cell Lung	6.6	
SK-Br-3	Breast	1.1	
MCF-7	Breast	6	
NCI-H1437	Non-small cell Lung	3.3	
HPAF-II	Pancreas	10.8	
Colo205	Colon	0.43-2.2*	0.2
Myla 2059	T cell Lymphoma	0.5	
MG63	Bone	0.6	0.3
PC-3	Prostate	0.8	0.8
T47D	Breast	0.79-2.53	
MDA-MB-231	Breast	10.9	
MDA-MB-468	Breast	1.1	
A549	Lung	3.5	1.8
253J B-V	Bladder	8.6	
UM-UC-3	Bladder	1.4	1.8
K562	Bone	8.8	

		EC_{50} (nM)	
		Toxin	
Cell Line	**Cancer Tissue Type**	**Compound O**	**MCvcPABC-3.90**
Rh30	Bone	0.6	
U2OS	Bone	10.3	
U138MG	Brain	0.2	
A172	Brain	1.9	
K562 #14	Control (Bone)	<100 nM	
K562 #16	Control (Bone)	<100 nM	
DU-145	Prostate	1.6	
MCF-7	Breast	2.4	
HepG2	Liver	15.1	
SK-OV-3	Ovary	4.4	
JIMT-1	Breast	2.3	
OE19	Oesophagus	3.7	

*EC_{50} is larger since these cells are used at 2 × the density of the others. EC_{50} ranged from 0.5-1 at 2500 cells/well.

Example 5: Tolerability Study

Study Outline

Female CD-1 mice (Harlan Laboratories) were injected with the test article VAR2-Compound O at a dose of 1.0 mg/kg q2dx3. Dose escalation or reductions following assessment of tolerability as outlined in the Study Grouping Table 8. Mice were weighed 3x weekly for 12 days.

Table 8. Study Grouping

Group #	Group Name	n	Admin. Route	Dose (mg/kg)	Dose Volume (mL/kg)	Dosing Schedule	Injection day
1	1.0	3	IV	1.0	10	q2dx3	1, 3, 5
2	0.3 or 3.0	3	IV	0.3 or 3.0*	10	q2dx3	1, 3, 5
3	0.1 or 9.0	3	IV	0.1 or 9.0**	10	q2dx3	1, 3, 5
4	6.0 or 15	3	IV	6.0 or 15***	10	q2dx3	1, 3, 5

* Dose to be determined after tolerability of group 1 is determined. If 1 mg/kg is well tolerated after 3 administrations, then 3 mg/kg will be tested. If 1 mg/kg is not well tolerated, then 0.3 mg/kg will be tested.

** Dose to be determined after tolerability of group 1 and group 2 is determined. If groups 1 and 2 are tolerated after 3 administrations, then 9 mg/kg will be tested. If groups 1 and 2 are not well tolerated after 3 administrations, then 0.1 mg/kg is tested. Otherwise, this group will be abandoned or an alternate dose will be selected.

*** Dose to be determined after tolerability of group 3 at 9 mg/kg is determined. If group 3 is tolerated after 3 administrations, then 15 mg/kg will be tested. If group 3 is not well tolerated after 3 administrations, then 6 mg/kg is tested.

Results

Treatment

All animals received their doses as indicated in the Injection Record.

Body Weights

No significant body weight loss was observed in any groups. Body Weights (Means ± S.D.) are shown in Figure 20.

Conclusions

For VAR2-Compound O experiments, no mice were terminated prior to scheduled sacrifice Day 12, indicating that the doses tested (up to 15 mg/kg) were tolerated.

Example 6: Karpas 299 Xenograft Efficacy Study

Study Overview

Female C.B-17/IcrHsd-Prkdcscid mice (Harlan Laboratories) were implanted subcutaneously in the back with the Karpas 299 human T cell lymphoma tumor cell line. Karpas 299 was established from the peripheral blood of a 25-year-old man with T cell non-Hodgkin's lymphoma in 1986; now classed as CD30+ anaplastic large cell lymphoma (ALCL). The lab stocks were mycoplasma negative. Tumors established over a period of 19 days, and test subjects were then grouped according to tumor volume such that each group (n=7) had an equal distribution of tumor volumes. The mean tumor volume on treatment day (day 21) was greater than 150 mm^3. Test articles were administered intravenously on Day 1, 3, and 6 (total of three injections) at the doses indicated in the study grouping table. Body weights and tumor volumes were measured every Monday, Wednesday, and Friday. Animals remained on study until their tumors reached 800 mm^3 in size or they otherwise required euthanasia due to achieving a humane endpoint.

Table 9. Study Grouping

Group #	Group Name	n	Admin. Route	Dose (mg/kg)	Dose Volume (mL/kg)	Schedule	Injection Days
1	Vehicle	7	IV	n/a	10	q2dx3	Wed, Fri, Mon
2	VAR2-Compound O	7	IV	12	10	q2dx3	Wed, Fri, Mon
3	VAR2	7	IV	12	10	q2dx3	Wed, Fri, Mon
4	Compound 886	7	IV	0.312	10	q2dx3	Wed, Fri, Mon

Results

Body weights of study mice following three IV doses of test articles are shown in Figure 21. Tumor volumes of study mice following three IV doses of test articles are shown in Figure 22. Statistical analysis of tumor volume by two-way ANOVA Bonferroni posttests are shown in Table 10.

Table 10

Study Day	Vehicle versus		
	VAR2-Compound O	VAR2	Compound 886
21	ns	ns	ns
23	ns	ns	ns
26	***	ns	*
28	***	ns	***
30	***	ns	***

Study Day	VAR2 versus		
	VAR2-Compound O	VAR2	Compound 886
21	ns	-	ns
23	ns	-	ns
26	*	-	ns
28	***	-	*
30	***	-	**

$P > 0.05$ = ns (not significant), $P < 0.05$ = *, $P < 0.01$ = **, $P < 0.001$ = ***

Conclusion

In conclusion, VAR2-Compound O inhibited the Karpas 299 tumor growth.

Example 7: PC3 Prostate Cancer Efficacy Study

Study Overview

Male nude nu/nu mice (Harlan Laboratories) were implanted subcutaneously in the back with the PC3 prostate cancer cell line in 100 µl of Matrigel® in both right and left flanks. The lab stocks were mycoplasma negative. Tumors established over a period of 28 days, and test subjects were grouped according to tumor volume such that each group had an equal distribution of tumor volumes. The mean tumor volume on treatment day was greater than 200 mm^3. Test articles were administered intravenously on Day 1, 3, and 6 (total of three injections) at the doses indicated in the study grouping table. Body weights and tumor volumes were measured every Monday, Wednesday, and Friday. Animals remained on study until their tumors reached 1000 mm^3 in size or they otherwise required euthanasia due to achieving a humane endpoint.

Table 11. Study Grouping

Group #	Group Name	N: Mice, Tumors	Admin. Route	Dose (mg/kg)	Dose Volume (mL/kg)	Schedule	Injection Days
1	Vehicle	7M (14T)	IV	n/a	10	q2dx3	Mon, Wed, Sat
2	VAR2-Compound O	8M (16T)	IV	12	10	q2dx3	Mon, Wed, Sat
3	VAR2	8M (15T)	IV	12	10	q2dx3	Mon, Wed, Sat
4	Compound 886	8M (16T)	IV	0.312	10	q2dx3	Mon, Wed, Sat

Results

Body Weights

The body weights of study mice are shown in Figure 23. No difference in body weight loss between the different arms of treatment. The observed body weight loss is due to tumor volume increase. No clinical toxicity signs were observed.

Tumor Volumes

Tumor volumes of study mice are shown in Figure 24. Data shows mean tumor volume and SEM (Tumor volume was calculated using the formula $V = 0.5 \times W \times L \times l$. Statistical evaluation performed using T-test as shown in Table 12.

Table 12

Time (days)	T-test p-values		
	Vehicle/ VAR2-Compound O	Compound 886/ VAR2-Compound O	VAR2/ VAR2-Compound O
-14	0.2931 (ns)	0.9583 (ns)	0.7097 (ns)
-7	0.5034 (ns)	0.3604 (ns)	0.6627 (ns)
-4	0.3161 (ns)	0.4445 (ns)	0.3808 (ns)
0	0.9817 (ns)	0.8854 (ns)	0.7867 (ns)
2	0.0812 (ns)	0.0801 (ns)	0.2466 (ns)
4	0.0394*	0.0918 (ns)	0.0961 (ns)
7	0.0036***	0.0065***	0.0004***
9	0.0026***	0.0060***	0.0001***
11	0.0025***	0.0228***	0.0003***
15	0.0016***	0.0035***	0.0001***
17	0.0007***	0.0003***	2.0626×10^{-5}***
19	0.0136*	0.0013***	3.20276×10^{-5}***

$P > 0.05$ = ns (not significant), $P < 0.05$ = *, $P < 0.001$ = ***

Necropsy

Mice were sacrificed when total tumor volume reached 1000 mm3 or when clinical signs were observed as body weight loss. Necropsy didn't show any toxicity or abnormality signs. The number of mice reaching endpoint is shown in Table 13

Table 13

Time (days)	Mice Reaching End Point		
	Vehicle	VAR2	VAR2-Compound O
-14	-	-	-
-7	-	-	-
-4	-	-	-
0	-	-	-
2	-	-	-
4	1	-	-

Time (days)	Mice Reaching End Point		
	Vehicle	VAR2	VAR2-Compound O
7	-	-	-
9	-	-	-
11	-	-	-
15	-	-	-
17	2	-	-
19	1	2	1

Conclusions

In conclusion, VAR2-Compound O inhibited the PC3 tumor growth. Twenty days after the last treatment, tumor volume average in VAR2-Compound O arm was still stable and was significantly lower than Vehicle, VAR2 and Compound 886 arms.

All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification are incorporated herein by reference, in their entirety to the extent not inconsistent with the present description. From the foregoing it will be appreciated that, although specific embodiments described herein have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope described herein. Accordingly, the disclosure is not limited except as by the appended claims.

It is contemplated that the different parts of the present description may be combined in any suitable manner. For instance, the present examples, methods, aspects, embodiments or the like may be suitably implemented or combined with any other embodiment, method, example or aspect of the invention.

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

Use of examples in the specification, including examples of terms, is for illustrative purposes only and is not intended to limit the scope and meaning of the embodiments of the invention herein. Numeric ranges are inclusive of the numbers defining the range. In the specification, the word "comprising" is used as an open-ended term, substantially equivalent to the phrase "including, but not limited to," and the word "comprises" has a corresponding meaning.

CLAIMS

1. A compound of Formula I:

T–L–P

I

wherein:

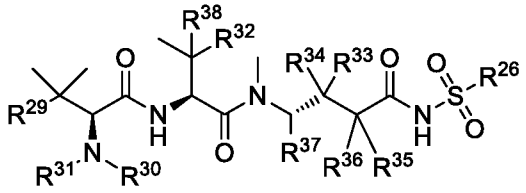
T is a targeting moiety comprising a VAR2CSA polypeptide, the VAR2CSA polypeptide comprising a sequential amino acid sequence of ID1 and DBL2Xb, and

L–P is L^1–P^1 or L^2–P^2;

wherein:

L^1 is a linker, or L^1 is absent;

P^1 is a monovalent radical of a compound of Formula XV:



XV

wherein:

R^{26} is selected from the group consisting of C_1-C_6 alkyl, aryl, aryl-C_1-C_6 alkyl, C_3-C_7 cycloalkyl, heteroaryl, and heterocyclyl, each optionally substituted with one or more substituents selected from: C_1-C_6 alkoxy, C_1-C_6 alkoxycarbonyl, C_1-C_6 alkyl, C_1-C_6 alkylamino, amino, amino-C_1-C_6 alkyl, amino-aryl, amino-C_3-C_7 cycloalkyl, aryl, carboxamide, carboxyl, C_3-C_7 cycloalkyl, cyano, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, halo, hydroxyl, nitro, thio, and thio-C_1-C_6 alkyl;

R^{29} is selected from the group consisting of aryl, C_3-C_7 cycloalkyl, and heteroaryl, each of which is optionally substituted with one or more substituents selected from: C_1-C_4 acylthio, C_2-C_4 alkenyl, C_1-C_4 alkyl, C_1-C_4 alkylamino, C_1-C_4 alkoxy, amino, amino-C_1-C_4 alkyl, halo, C_1-C_4 haloalkyl, hydroxyl, hydroxy-C_1-C_4 alkyl, and thio, wherein C_2-C_4 alkenyl, C_1-C_4 alkylamino and C_1-C_4 alkoxy are

further optionally substituted with one substituent selected from C_1-C_4 alkylaryl, hydroxyl, and thio;

R^{30} is selected from the group consisting of H and C_{1-6} alkyl;

R^{31} is selected from the group consisting of H and C_{1-6} alkyl;

R^{32} and R^{38} are each independently selected from the group consisting of H, C_{1-6} alkyl and –SH, with the proviso that R^{32} and R^{38} cannot both be H;

R^{33}, R^{34}, R^{35} and R^{36} are each independently H or C_{1-6} alkyl, wherein at least one of R^{33} and R^{34} is H; or R^{34} and R^{35} form a double bond, R^{33} is H, and R^{36} is H or C_{1-6} alkyl; and

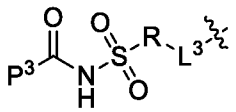
R^{37} is selected from the group consisting of H and C_{1-6} alkyl; and

wherein:

L^2 is a linker;

P^2 is a cytotoxic compound selected from a hemiasterlin, a hemiasterlin analog, a tubulysin, a tubulysin analog, an auristatin, and an auristatin analog; and

L^2–P^2 has the following structure (III):



III

wherein:

R is selected from the group consisting of C_1-C_6 alkyl, aryl, aryl-C_1-C_6 alkyl, C_3-C_7 cycloalkyl, heteroaryl, and heterocyclyl, each optionally substituted with one or more substituents selected from: C_1-C_6 alkoxy, C_1-C_6 alkoxycarbonyl, C_1-C_6 alkyl, C_1-C_6 alkylamino, amino, amino-C_1-C_6 alkyl, amino-aryl, amino-C_3-C_7 cycloalkyl, aryl, carboxamide, carboxyl, C_3-C_7 cycloalkyl, cyano, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, halo, hydroxyl, nitro, thio, and thio-C_1-C_6 alkyl, or R is absent;

P^3 is the remaining portion of compound P^2; and

L^3 is the remaining portion of linker L^2 or is absent.

2. The compound of claim 1, wherein the VAR2CSA polypeptide comprises a sequential amino acid sequence of:

a. ID1;

b. DBL2Xb; and

c. ID2a.

3. The compound of claim 2, wherein the VAR2CSA polypeptide further comprises an amino acid sequence at the N- or C- terminus of 100 amino acids or less derived from a part of SEQ ID NO: 55 or SEQ ID NO: 56, wherein the part is not 1D1, DBL2Xb or ID2a.

4. The compound of claim 1 or 2, wherein the VAR2CSA polypeptide comprises a sequential amino acid sequence of DBL1-ID2a.

5. The compound of any one of claims 1 to 4, wherein the VAR2CSA polypeptide binds chondroitin sulfate A (CSA) on proteoglycans (CSPG) with an affinity as measured by a K_D lower than 100 nM.

6. The compound of claim 1, wherein the VAR2CSA polypeptide comprises an amino acid sequence having at least 70% sequence identity with an amino acid sequence selected from: amino acids 1-577 of SEQ ID NO:1; amino acids 1-592 of SEQ ID NO:3; amino acids 1-579 of SEQ ID NO:4; amino acids 1-576 of SEQ ID NO:5; amino acids 1-586 of SEQ ID NO:10; amino acids 1-579 of SEQ ID NO:11; amino acids 1-565 of SEQ ID NO:29; amino acids 1-584 of SEQ ID NO:34; amino acids 1-569 of SEQ ID NO:36; amino acids 1-575 of SEQ ID NO:37; amino acids 1-592 of SEQ ID NO:38; amino acids 1-603 of SEQ ID NO:41; amino acids 1-588 of SEQ ID NO:43; amino acids 1-565 of SEQ ID NO:44; amino acids 1-589 of SEQ ID NO:45; amino acids 1-573 of SEQ ID NO:48; amino acids 1-583 of SEQ ID NO:53; amino acids 1-569 of SEQ ID NO:54; amino acids 578-640 of SEQ ID NO:1; amino acids 593-656 of SEQ ID NO:3; amino acids 580-643 of SEQ ID NO:4; amino acids 577-

640 of SEQ ID NO:5; amino acids 587-650 of SEQ ID NO:10; amino acids 580-643 of SEQ ID NO:11; amino acids 566-628 of SEQ ID NO:29; amino acids 585-647 of SEQ ID NO:34; amino acids 570-632 of SEQ ID NO:36; amino acids 576-639 of SEQ ID NO:37; amino acids 593-655 of SEQ ID NO:38; amino acids 604-667 of SEQ ID NO:41; amino acids 589-652 of SEQ ID NO:43; ~~30~~ amino acids 566-628 of SEQ ID NO:44; amino acids 590-653 of SEQ ID NO:45; amino acids 574-637 of SEQ ID NO:48; amino acids 584-646 of SEQ ID NO:53; amino acids 570-632 of SEQ ID NO:54; SEQ ID NO:2; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; SEQ ID NO:15; SEQ ID NO:16; SEQ ID NO:17; SEQ ID NO:18; SEQ ID NO:19; SEQ ID NO:20; SEQ ID NO:21; SEQ ID NO:22; SEQ ID NO:23; SEQ ID NO:24; SEQ ID NO:25; SEQ ID NO:26; SEQ ID NO:27; SEQ ID NO:28; SEQ ID NO:30; SEQ ID NO:31; SEQ ID NO:32; SEQ ID NO:33; SEQ ID NO:35; SEQ ID NO:39; SEQ ID NO:40; SEQ ID NO:42; SEQ ID NO:46; SEQ ID NO:47; SEQ ID NO:49; SEQ ID NO:50; SEQ ID NO:51; SEQ ID NO:52; SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:29; SEQ ID NO:34; SEQ ID NO:36; SEQ ID NO:37; SEQ ID NO:38; SEQ ID NO:41; SEQ ID NO:43; SEQ ID NO:44; SEQ ID NO:45; SEQ ID NO:48; SEQ ID NO:53; and SEQ ID NO:54.

7. The compound of claim 1, wherein the VAR2CSA polypeptide comprises an amino acid sequence having at least 70% sequence identity with an amino acid sequence selected from: amino acids 1-577 of SEQ ID NO:1; amino acids 1-592 of SEQ ID NO:3; amino acids 1-579 of SEQ ID NO:4; amino acids 1-576 of SEQ ID NO:5; amino acids 1-586 of SEQ ID NO:10; amino acids 1-579 of SEQ ID NO:11; amino acids 1-565 of SEQ ID NO:29; amino acids 1-584 of SEQ ID NO:34; amino acids 1-569 of SEQ ID NO:36; amino acids 1-575 of SEQ ID NO:37; amino acids 1-592 of SEQ ID NO:38; amino acids 1-603 of SEQ ID NO:41; amino acids 1-588 of SEQ ID NO:43; amino acids 1-565 of SEQ ID NO:44; amino acids 1-589 of SEQ ID NO:45; amino acids 1-573 of SEQ ID NO:48; amino acids 1-583 of SEQ ID NO:53; amino acids 1-569 of SEQ ID NO:54; SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:4; SEQ

ID NO:5; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:29; SEQ ID NO:34; SEQ ID NO:36; SEQ ID NO:37; SEQ ID NO:38; SEQ ID NO:41; SEQ ID NO:43; SEQ ID NO:44; SEQ ID NO:45; SEQ ID NO:48; SEQ ID NO:53; and SEQ ID NO:54.

8. The compound of any one of claims 1 to 4, wherein the VAR2CSA polypeptide consists of an amino acid sequence having a length of less than 700 amino acids.

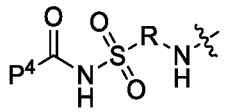
9. The compound of any one of claims 1 to 8, wherein the VAR2CSA polypeptide is a recombinant protein.

10. The compound of any one of claims 1 to 9, wherein the VAR2CSA polypeptide is non-glycosylated.

11. The compound of any one of claims 1 to 10, wherein L–P is L^2–P^2.

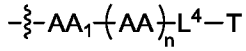
12. The compound of claim 11, wherein:

P^2 is a compound of Formula V:



V

L^2–T has the following structure (VI):



VI

wherein

P^4 is the remaining portion of compound P^2,

the –NH– group bonded to R in Formula V forms a peptide bond (JPB) with AA_1 in formula VI, wherein the JPB is enzymatically cleavable,

R is selected from the group consisting of C_1-C_6 alkyl, aryl, aryl-C_1-C_6 alkyl, C_3-C_7 cycloalkyl, heteroaryl, and heterocyclyl, each optionally substituted with one or more

substituents selected from: C_1-C_6 alkoxy, C_1-C_6 alkoxycarbonyl, C_1-C_6 alkyl, C_1-C_6 alkylamino, amino, amino-C_1-C_6 alkyl, amino-aryl, amino-C_3-C_7 cycloalkyl, aryl, carboxamide, carboxyl, C_3-C_7 cycloalkyl, cyano, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, halo, hydroxyl, nitro, thio, and thio-C_1-C_6 alkyl,

each AA is independently an amino acid,

n is an integer from 0 to 25,

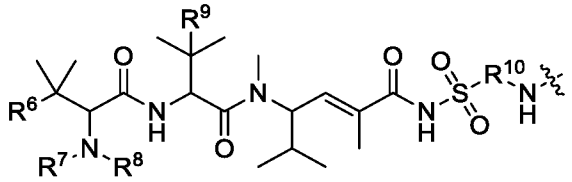
L^4 is the remaining portion of linker L^2 or is absent, and

T is the targeting moiety, and

wherein AA_1–$(AA)_n$, taken together comprises an amino acid sequence capable of facilitating enzymatic cleavage of the JPB.

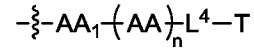
13. The compound of claim 11 or 12, wherein the cytotoxic compound is a hemiasterlin, a hemiasterlin analog, an auristatin, or an auristatin analog.

14. The compound of claim 11, wherein P^2 has the following structure (X):



X

and L^2–T has the following structure (IV):



VI

wherein:

R^6 is selected from: aryl, C_3-C_7 cycloalkyl, and heteroaryl, each of which is optionally substituted with one or more substituents selected from: C_1-C_4 acylthio, C_2-C_4 alkenyl, C_1-C_4 alkyl, C_1-C_4 alkylamino, C_1-C_4 alkoxy, amino, amino-C_1-C_4 alkyl, halo, C_1-C_4 haloalkyl, hydroxyl, hydroxy-C_1-C_4 alkyl, and thio, wherein C_2-C_4 alkenyl, C_1-C_4 alkylamino and C_1-C_4 alkoxy are further optionally substituted with one substituent selected from C_1-C_4 alkylaryl, hydroxyl, and thio;

R^7 and R^8 are each independently H or C_1-C_6 alkyl;

R^9 is C_1-C_6 alkyl or thio;

R^{10} is selected from C_1-C_6 alkyl, aryl, aryl-C_1-C_6 alkyl, C_3-C_7 cycloalkyl, heteroaryl, and heterocyclyl, each optionally substituted with one or more substituents selected from: C_1-C_6 alkoxy, C_1-C_6 alkoxycarbonyl, C_1-C_6 alkyl, C_1-C_6 alkylamino, amino, amino-C_1-C_6 alkyl, amino-aryl, amino-C_3-C_7 cycloalkyl, aryl, carboxamide, carboxyl, C_3-C_7 cycloalkyl, cyano, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, halo, hydroxyl, nitro, thio, and thio-C_1-C_6 alkyl;

each AA is independently an amino acid;

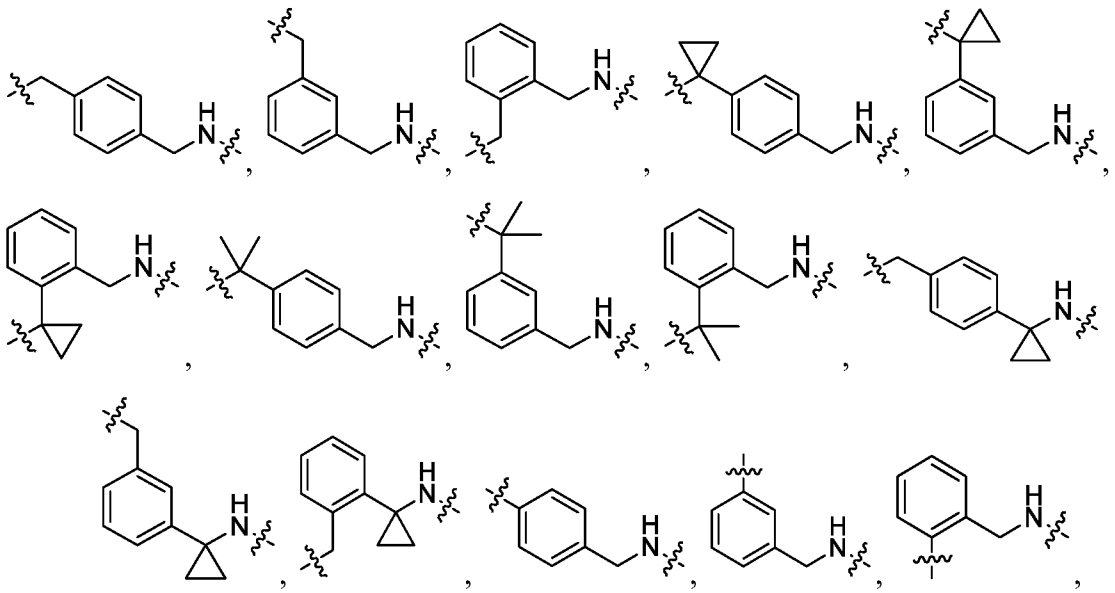
n is an integer from 0 to 25;

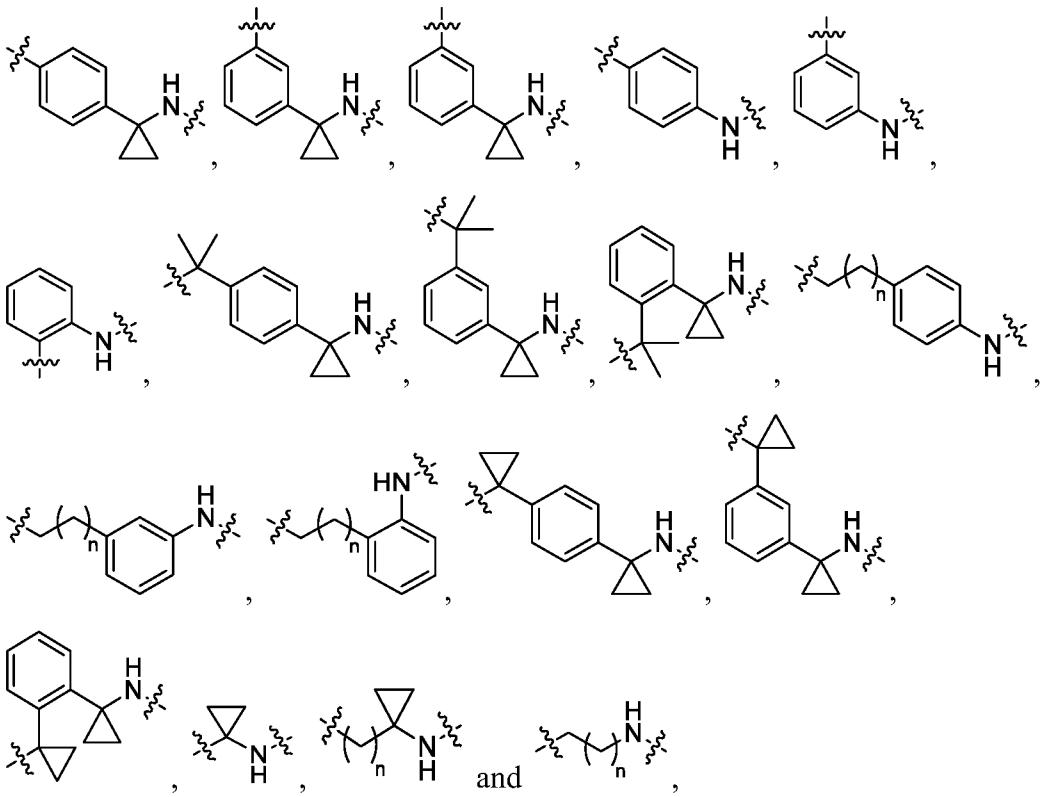
L^4 is the remaining portion of linker L^2 or is absent;

T is the targeting moiety;

wherein the –NH– group bonded to R^{10} in Formula X forms a junction peptide bond (JPB) with AA_1 in Formula VI, wherein the JPB is enzymatically cleavable, and wherein AA_1–$(AA)_n$, taken together comprises an amino acid sequence capable of facilitating enzymatic cleavage of the JPB.

15. The compound of claim 14, wherein –R^{10}–NH– of Formula X is selected from:





and

wherein each n is independently an integer from 0-10.

16. The compound of claim 11, wherein P^2 is a monovalent radical of one of the following compounds:

a) (S,E)-N-(3-Mercaptopropylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound A);

b) (S,E)-N-(2-Mercaptoethylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound B);

c) (S,E)-N-(4-(Mercaptomethyl)phenylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound C);

d) 4-(N-((S,E)-2,5-Dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enoyl)sulfamoyl)-3-nitrobenzamide;

e) (S,E)-N-(4-Aminophenylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound 886);

f) 4-(N-((S,E)-2,5-Dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enoyl)sulfamoyl)benzoic acid;

g) (S,E)-N-(3-Aminophenylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

h) (S,E)-N-(4-(1-Aminocyclopropyl)benzylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

i) (S,E)-N-(4-(1-Aminocyclopropyl)phenylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

j) (S,E)-N-(4-(Aminomethyl)benzylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

k) (S,E)-N-(4-Aminobenzylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

l) (S,E)-N-(4-(Aminomethyl)phenylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

m) (S,E)-N-(2-Aminophenylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

n) (S,E)-N-(4'-Aminobiphenyl-4-ylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

o) (S,E)-N-(4-Amino-2-ethylphenylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

p) (S,E)-N-(4-Amino-3-(trifluoromethoxy)phenylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

q) (S,E)-N-(4-Amino-2,3-dimethylphenylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

r) (S,E)-N-(4-Amino-5,6,7,8-tetrahydronaphthalen-1-ylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

s) (S,E)-N-(4-Amino-3-methylphenylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

t) (S,E)-N-(4-Amino-3-fluorophenylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

u) (S,E)-N-(4-Amino-3-ethylphenylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

v) (S,E)-N-(4-Amino-3-(trifluoromethyl)phenylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

w) (S)-1-Isopropyl-N-((S)-1-(((S,E)-6-(3-mercaptopropylsulfonamido)-2,5-dimethyl-6-oxohex-4-en-3-yl)(methyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)piperidine-2-carboxamide;

x) (S)-N-((S)-1-((S)-2-((E)-3-(3-Mercaptopropylsulfonamido)-2-methyl-3-oxoprop-1-enyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-3-methyl-2-(methylamino)-3-phenylbutanamide;

y) (S)-N-((S)-1-(2-(3-(3-Mercaptopropylsulfonamido)-2-methyl-3-oxoprop-1-enyl)piperidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-3-methyl-2-(methylamino)-3-phenylbutanamide; or

z) (S)-N-((S)-1-(2-(3-(4-(Mercaptomethyl)phenylsulfonamido)-2-methyl-3-oxoprop-1-enyl)piperidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-3-methyl-2-(methylamino)-3-phenylbutanamide.

17. The compound of claim 12, wherein P^2 is a monovalent radical of one of the following compounds:

a) (S,E)-N-(4-Aminophenylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound 886);

b) (S,E)-N-(4-(1-Aminocyclopropyl)benzylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

c) (S,E)-N-(4-(1-Aminocyclopropyl)phenylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

d) (S,E)-N-(4-(Aminomethyl)benzylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

e) (S,E)-N-(4-Aminobenzylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide; or

f) (S,E)-N-(4-(Aminomethyl)phenylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

18. The compound of any one of claims 12 to 15 and 17, wherein $AA_1–(AA)_n$ is Phe-Lys, Val-Lys, Ala-Lys, Val-Cit, Phe-Cit, Leu-Cit, Ile-Cit, Trp-Cit, Phe-Arg, (D)Phe-Phe-Lys or (D)Ala-Phe-Lys.

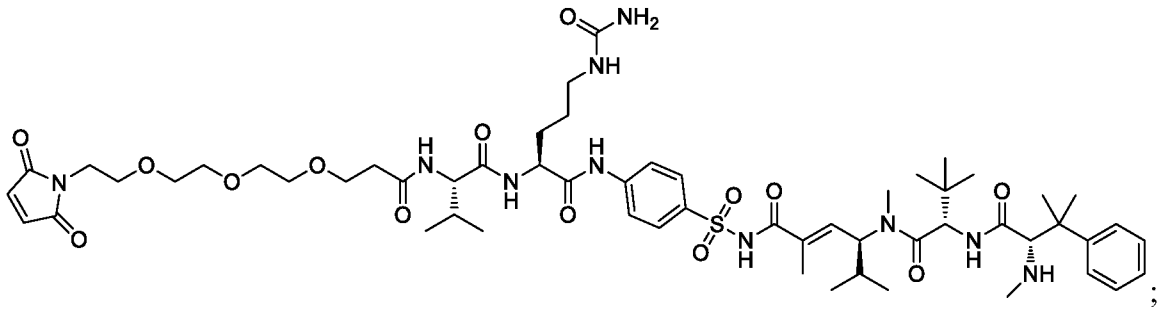
19. The compound of any one of claims 12 to 15, 17 and 18, wherein L^4 comprises repeating alkoxy units, a diacid amide, a diacid ester, or a combination thereof.

20. The compound of claim 12, wherein L^2-P^2 is a monovalent radical of one of the following compounds:

a) (S,E)-N-(4-((S)-2-((S)-2-(6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)phenylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound N);

or

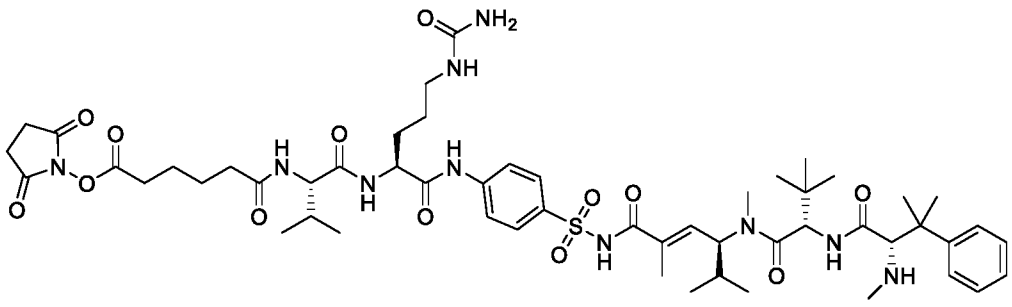
b) Compound O:



;

or

c) Compound KK:

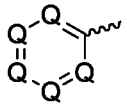


.

21. The compound of any one of claims 1 to 10, wherein L–P is L^1–P^1.

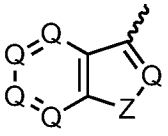
22. The compound of claim 21, wherein each aryl and heteroaryl is, independently, selected from the group consisting of phenyl, naphthyl, anthracyl, phenanthryl, furyl, pyrrolyl, thiophenyl, benzofuryl, benzothiophenyl, quinolinyl, isoquinolinyl, imidazolyl, thiazolyl, oxazolyl, and pyridinyl.

23. The compound of claim 21, wherein R^{29} is selected from one of the following structures XVI, XVII, XVIII, and XIX:



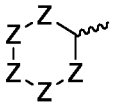
;

XVI



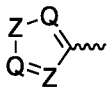
;

XVII



; and

XVIII



,

XIX

wherein:

Q is CR^{39} or N;

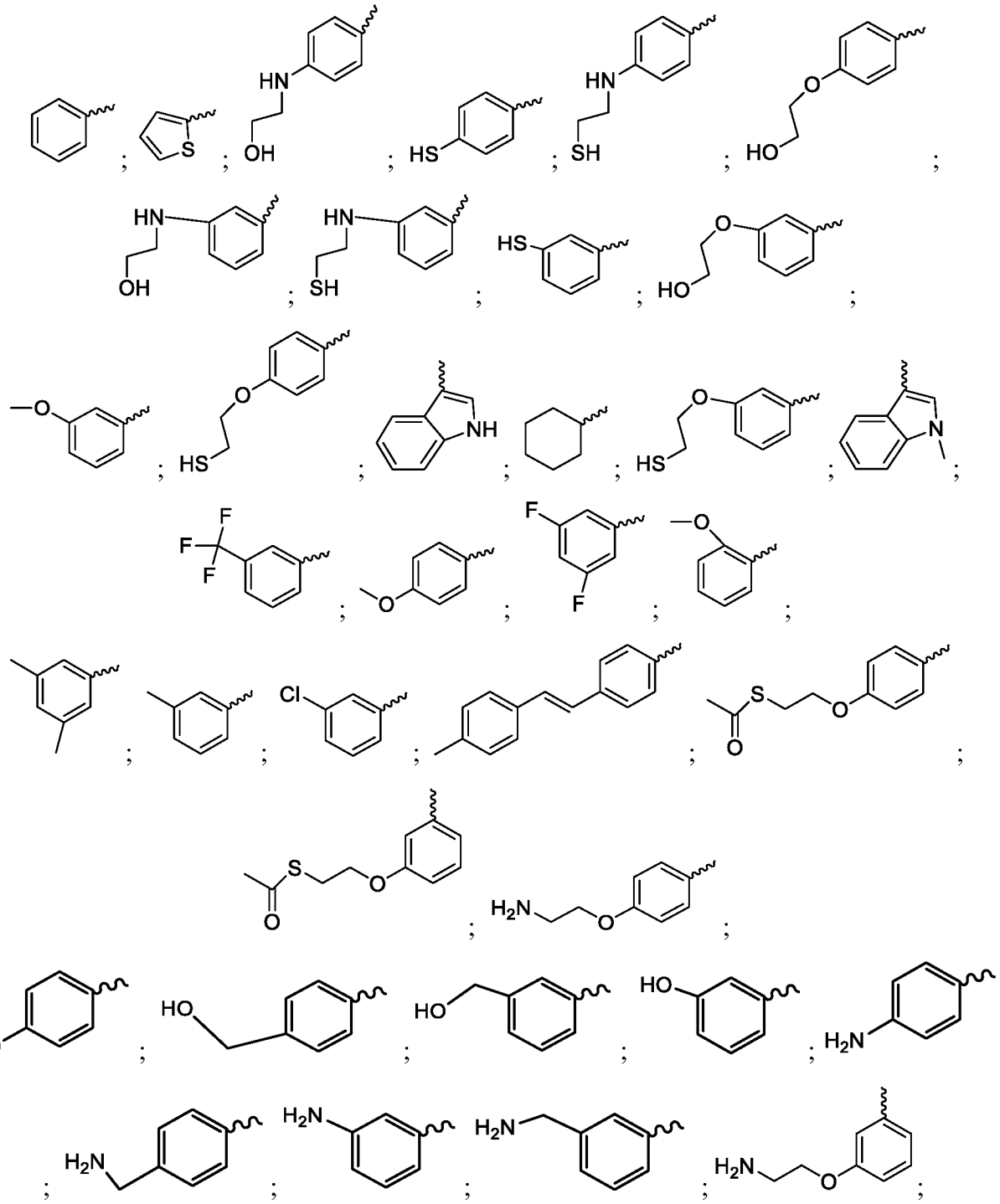
Z is $C(R^{39})_2$, NR^{39}, S, or O;

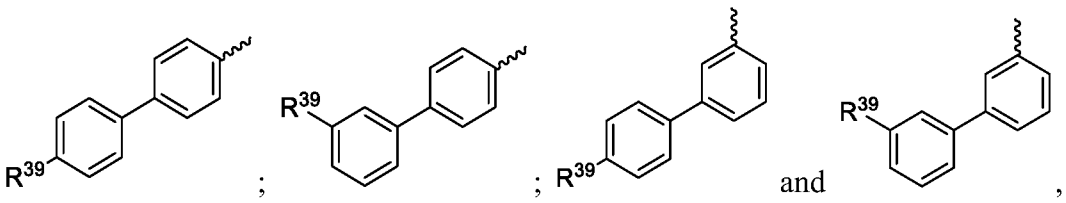
wherein in structure XIX, one instance of Z is CR^{39} or N, and the other instance is $(CR^{39})_2$, NR^{39}, S or O; and

each R^{39} is, independently, selected from the group consisting of H, –OH, $–R^{27}$, $–OR^{27}$, $–O_2CR^{27}$, –SH, $–SR^{27}$, $–SOCR^{27}$, $–NH_2$, $–N_3$, $–NHR^{27}$, $–N(R^{27})_2$, $–NHCOR^{27}$, $–NR^{27}COR^{27}$, $–R^{27}NH_2$, –I, –Br, –Cl, –F, –CN, $–CO_2H$, $–CO_2R^{27}$, –CHO, $–COR^{27}$, $–CONH_2$, $–CONHR^{27}$, $–CON(R^{27})_2$, –COSH, $–COSR^{27}$, $–NO_2$,

$-SO_3H$, $-SOR^{27}$, and $-SO_2R^{27}$, wherein each R^{27} is, independently, alkyl optionally substituted with halogen, –OH or –SH.

24. The compound of claim 21, wherein R^{29} is selected from the group consisting of:

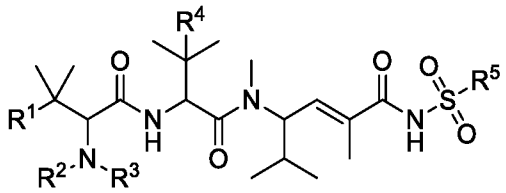




wherein each R^{39} is independently selected from the group consisting of H, –OH, $–R^{27}$, $–OR^{27}$, $–O_2CR^{27}$, –SH, $–SR^{27}$, $–SOCR^{27}$, $–NH_2$, $–N_3$, $–NHR^{27}$, $–N(R^{27})_2$, $–NHCOR^{27}$, $–NR^{27}COR^{27}$, $–R^{27}NH_2$, –I, –Br, –Cl, –F, –CN, $–CO_2H$, $–CO_2R^{27}$, –CHO, $–COR^{27}$, $–CONH_2$, $–CONHR^{27}$, $–CON(R^{27})_2$, –COSH, $–COSR^{27}$, $–NO_2$, $–SO_3H$, $–SOR^{27}$, and $–SO_2R^{27}$, wherein each R^{27} is, independently, alkyl optionally substituted with halogen, –OH or –SH.

25. The compound of any one of claims 21 to 24, wherein R^{30} is H or methyl, and R^{31}, R^{32}, and R^{38} are each methyl.

26. The compound of claim 21, wherein P^1 is a monovalent radical of a compound of Formula II:



II

wherein:

R^1 is selected from: aryl, C_3-C_7 cycloalkyl, and heteroaryl, each of which is optionally substituted with one or more substituents selected from: C_1-C_4 acylthio, C_2-C_4 alkenyl, C_1-C_4 alkyl, C_1-C_4 alkylamino, C_1-C_4 alkoxy, amino, amino-C_1-C_4 alkyl, halo, C_1-C_4 haloalkyl, hydroxyl, hydroxy-C_1-C_4 alkyl, and thio, wherein C_2-C_4 alkenyl, C_1-C_4 alkylamino and C_1-C_4 alkoxy are further optionally substituted with one substituent selected from C_1-C_4 alkylaryl, hydroxyl, and thio;

R^2 and R^3 are each independently H or C_1-C_6 alkyl;

R^4 is C_1-C_6 alkyl or thio; and

R^5 is selected from: C_1-C_6 alkyl, aryl, aryl-C_1-C_6 alkyl, C_3-C_7 cycloalkyl, heteroaryl, and heterocyclyl, each optionally substituted with one or more substituents selected from: C_1-C_6 alkoxy, C_1-C_6 alkoxycarbonyl, C_1-C_6 alkyl, C_1-C_6 alkylamino, amino, amino-C_1-C_6 alkyl, amino-aryl, amino-C_3-C_7 cycloalkyl, aryl, carboxamide, carboxyl, C_3-C_7 cycloalkyl, cyano, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, halo, hydroxyl, nitro, thio, and thio-C_1-C_6 alkyl.

27. The compound of claim 26, wherein R^1 is selected from: 1*H*-indol-3-yl, 1-methyl-1*H*-indol-3-yl, 2-methoxyphenyl, 3-((2-hydroxyethyl)amino)phenyl, 3-((2-mercaptoethyl)amino)phenyl, 3-(2-(acetylthio)ethoxy)phenyl, 3-(2-hydroxyethoxy)phenyl, 3-(2-mercaptoethoxy)phenyl, 3-(4-methylstyryl)phenyl, 3-(aminomethyl)phenyl, 3-(hydroxymethyl)phenyl, 3-hydroxyphenyl, 3,5-difluorophenyl, 3,5-dimethylphenyl, 3-aminophenyl, 3-chlorophenyl, 3-mercaptophenyl, 3-methoxyphenyl, 3-trifluoromethylphenyl, 4-((2-hydroxyethyl)amino)phenyl, 4-((2-mercaptoethyl)amino)phenyl, 4-(2-(acetylthio)ethoxy)phenyl, 4-(2-aminoethoxy)phenyl, 4-(2-hydroxyethoxy)phenyl, 4-(2-mercaptoethoxy)phenyl, 4-(aminomethyl)phenyl, 4-(hydroxymethyl)phenyl, 4-aminophenyl, 4-hydroxyphenyl, 4-mercaptophenyl, 4-methoxyphenyl, cyclohexyl, thien-2-yl, *m*-tolyl, and phenyl.

28. The compound of claim 26 or 27, wherein R^2 is H, or methyl, and/or R^3 is methyl, and/or R^4 is methyl.

29. The compound of any one of claims 26 to 28, wherein R^5 is selected from: C_1-C_6 alkyl, aryl, aryl-C_1-C_6 alkyl, C_3-C_7 cycloalkyl, heteroaryl, and heterocyclyl, each optionally substituted with one or more substituents selected from: 1-aminocyclopropyl, 4-aminophenyl, amino, aminomethyl, bromo, *tert*-butyl, carboxamide, carboxyl, chloro, cyano, cyclopentyl, ethyl, fluoro, hydroxy, isopropyl,

methoxy, methyl, nitro, phenyl, pyridin-3-yl, thio, thiomethyl, trifluoromethoxy, and trifluoromethyl.

30. The compound of any one of claims 26 to 28, wherein R^5 is selected from: 4-aminobenzyl, 4-(aminomethyl)benzyl, 4-(aminomethyl)phenyl, 4-aminophenyl, benzyl, 3-mercaptopropyl, 2-mercaptoethyl, 4-(mercaptomethyl)phenyl, *p*-tolyl, methyl, 2,4,6-trimethylphenyl, 4-(trifluoromethoxy)phenyl, 2,4,6-triisopropylphenyl, 4-*tert*-butylphenyl, 4-chlorophenyl, 3-cyanophenyl, 2-nitrophenyl, 4-methoxy-2-nitrophenyl, 4-aminocarbonyl-2-nitrophenyl, 4-methoxyphenyl, 4-aminophenyl, phenyl, 2-fluorobenzyl, piperidin-1-yl, *o*-tolyl, 4-bromophenyl, naphthalen-2-yl, 4-methoxycarbonyphenyl, 2-(trifluoromethyl)benzyl, hexan-2-yl, 2-methoxyethyl, cyclopentylmethyl, cyclohexyl, pyridin-3-ylmethyl, 4-carboxyphenyl, 3-aminophenyl, pyridin-3-yl, thien-2-yl, 4-hydroxyphenyl, 4-(1-aminocyclopropyl)benzyl, 4-(1-aminocyclopropyl)phenyl, 2-methylbenzyl, 4-nitrobenzyl, 4-chlorobenzyl, phenethyl, 4-bromobenzyl, 4-cyanobenzyl, 3-nitrobenzyl, 4-*tert*-butylbenzyl, 2-nitrobenzyl, 4-nitrophenethyl, 2-chloro-3-methoxycarbonylphenyl, 2-aminophenyl, [1,1'-biphenyl]-4-yl, 4'-amino-[1,1'-biphenyl]-4-yl, 4-fluorobenzyl, 3-(trifluoromethyl)benzyl, 3-(trifluoromethoxy)benzyl, 3,4-dichlorobenzyl, 2-cyanobenzyl, 3-chlorobenzyl, 4-amino-2-ethylphenyl, 4-amino-3-(trifluoromethoxy)phenyl, 4-amino-2,3-dimethylphenyl, 4-amino-5,6,7,8-tetrahydronaphthalen-1-yl, 4-amino-3-methylphenyl, 4-amino-3-fluorophenyl, 4-amino-3-ethylphenyl, and 4-amino-3-(trifluoromethyl)phenyl.

31. The compound of claim 21, wherein P^1 is a monovalent radical of one of the following compounds:

a) (*S*,*E*)-*N*-(3-Mercaptopropylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound A);

b) (*S*,*E*)-*N*-(2-Mercaptoethylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound B);

c) *S*,*E*)-*N*-(4-(Mercaptomethyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound C);

d) (*S*,*E*)-2,5-Dimethyl-*N*-tosyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound D);

e) (*S*,*E*)-2,5-dimethyl-*N*-(methylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound E);

f) (*S*,*E*)-*N*-(Mesitylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

g) (*S*,*E*)-2,5-Dimethyl-*N*-(4-(trifluoromethoxy)phenylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

h) (*S*,*E*)-*N*-(Benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound 14);

i) (*S*,*E*)-2,5-Dimethyl-*N*-(2,4,6-triisopropylphenylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

j) (*S*,*E*)-*N*-(4-*tert*-Butylphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

k) (*S*,*E*)-*N*-(4-Chlorophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

l) (*S*,*E*)-*N*-(3-Cyanophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

m) (*S*,*E*)-2,5-Dimethyl-*N*-(2-nitrophenylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

n) (*S*,*E*)-*N*-(4-Methoxy-2-nitrophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

o) 4-(*N*-((*S*,*E*)-2,5-Dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enoyl)sulfamoyl)-3-nitrobenzamide;

p) (*S*,*E*)-*N*-(4-Methoxyphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

q) (*S*,*E*)-*N*-(4-Aminophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide (Compound 886);

r) (*S*,*E*)-2,5-Dimethyl-*N*-(phenylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

s) (*S*,*E*)-*N*-(*N*-(2-Fluorobenzyl)sulfamoyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

t) (*S*,*E*)-2,5-Dimethyl-*N*-(piperidin-1-ylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

u) (*S*,*E*)-2,5-Dimethyl-*N*-(o-tolylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

v) (*S*,*E*)-*N*-(4-Bromophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

w) (*S*,*E*)-2,5-Dimethyl-*N*-(naphthalen-2-ylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

x) Methyl 4-(*N*-((*S*,*E*)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enoyl)sulfamoyl)benzoate;

y) (*S*,*E*)-2,5-Dimethyl-*N*-(*N*-(2-(trifluoromethyl)benzyl)sulfamoyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

z) (4*S*,*E*)-*N*-(Hexan-2-ylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

aa) (*S*,*E*)-*N*-(2-Methoxyethylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

bb) (*S*,*E*)-*N*-(Cyclopentylmethylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

cc) (*S*,*E*)-*N*-(Benzylsulfonyl)-4-((*S*)-2-((*S*)-3-(4-cyanophenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enamide;

dd) (*S*,*E*)-4-((*S*)-2-((*S*)-3-(4-(Aminomethyl)phenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-*N*-(benzylsulfonyl)-2,5-dimethylhex-2-enamide;

ee) (*S*,*E*)-4-((*S*)-2-((*S*)-3-(4-Azidophenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-*N*-(benzylsulfonyl)-2,5-dimethylhex-2-enamide;

ff) (*S*,*E*)-4-((*S*)-2-((*S*)-3-(4-Aminophenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-*N*-(benzylsulfonyl)-2,5-dimethylhex-2-enamide;

gg) (*S*,*E*)-*N*-(Cyclohexylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

hh) 4-(*N*-((*S*,*E*)-2,5-Dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enoyl)sulfamoyl)benzoic acid;

ii) (*S*,*E*)-*N*-(3-Aminophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

jj) (*S*,*E*)-2,5-Dimethyl-*N*-(pyridin-3-ylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

kk) (*S*,*E*)-2,5-Dimethyl-*N*-(thiophen-2-ylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

ll) (*S*,*E*)-*N*-(4-Hydroxyphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

mm) (S,E)-N-(4-(1-Aminocyclopropyl)benzylsulfonyl)-2,5-dimethyl-4-((S)-N,3,3-trimethyl-2-((S)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

nn) (*S*,*E*)-*N*-(4-(1-Aminocyclopropyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

oo) (*S*,*E*)-2,5-Dimethyl-*N*-(2-methylbenzylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

pp) (*S*,*E*)-2,5-Dimethyl-*N*-(4-nitrobenzylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

qq) (*S*,*E*)-*N*-(4-Chlorobenzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

rr) (*S*,*E*)-2,5-Dimethyl-*N*-(phenethylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

ss) (*S*,*E*)-*N*-(4-Bromobenzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

tt) (*S*,*E*)-*N*-(4-Cyanobenzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

uu) (*S*,*E*)-2,5-Dimethyl-*N*-(3-nitrobenzylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

vv) (*S*,*E*)-*N*-(4-*tert*-Butylbenzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

ww) (*S*,*E*)-2,5-Dimethyl-*N*-(2-nitrobenzylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

xx) (*S*,*E*)-2,5-Dimethyl-*N*-(4-nitrophenethylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

yy) Methyl 4-Chloro-3-(*N*-((*S*,*E*)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enoyl)sulfamoyl)benzoate;

zz) (*S*,*E*)-*N*-(4-(Aminomethyl)benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

aaa) (*S*,*E*)-*N*-(4-Aminobenzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

bbb) (*S*,*E*)-*N*-(4-(Aminomethyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

ccc) (*S*,*E*)-*N*-(Benzylsulfonyl)-4-((*S*)-2-((*S*)-3-(4-bromophenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enamide;

ddd) (*S*,*E*)-*N*-(Benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-(4-(4-methylstyryl)phenyl)butanamido)butanamido)hex-2-enamide;

eee) (*S*,*E*)-*N*-(Benzylsulfonyl)-4-((*S*)-2-((*S*)-3-(4-methoxyphenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enamide;

fff) (*S*,*E*)-*N*-(Benzylsulfonyl)-4-((*S*)-2-((R)-3-(3-methoxyphenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enamide;

ggg) (*S*,*E*)-*N*-(Benzylsulfonyl)-4-((*S*)-2-((*S*)-3-(3-methoxyphenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enamide;

hhh) (*S*,*E*)-*N*-(Benzylsulfonyl)-4-((*S*)-2-((*S*)-3-(4-(2-hydroxyethoxy)phenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enamide;

iii) (*S*,*E*)-*N*-(2-Aminophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

jjj) (*S*,*E*)-*N*-(Biphenyl-4-ylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

kkk) (*S*,*E*)-*N*-(4'-Aminobiphenyl-4-ylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

lll) (*S*,*E*)-*N*-(4-Fluorobenzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

mmm) (*S*,*E*)-2,5-Dimethyl-*N*-(3-(trifluoromethyl)benzylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

nnn) (*S*,*E*)-2,5-Dimethyl-*N*-(3-(trifluoromethoxy)benzylsulfonyl)-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

ooo) (*S*,*E*)-*N*-(3,4-Dichlorobenzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

ppp) (*S*,*E*)-*N*-(2-Cyanobenzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

qqq) (*S*,*E*)-*N*-(3-Chlorobenzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

rrr) (*S*,*E*)-*N*-(4-Amino-2-ethylphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

sss) (*S*,*E*)-*N*-(4-Amino-3-(trifluoromethoxy)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

ttt) (*S*,*E*)-*N*-(4-Amino-2,3-dimethylphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

uuu) (*S*,*E*)-*N*-(4-Amino-5,6,7,8-tetrahydronaphthalen-1-ylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

vvv) (*S*,*E*)-*N*-(4-Amino-3-methylphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

www) (*S*,*E*)-*N*-(4-Amino-3-fluorophenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

xxx) (*S*,*E*)-*N*-(4-Amino-3-ethylphenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

yyy) (*S*,*E*)-*N*-(4-Amino-3-(trifluoromethyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

zzz) (*R*)-*N*-(Benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hexanamide; or

aaaa) (*S*,*E*)-*N*-(Benzylsulfonyl)-4-((*S*)-2-((*S*)-3-cyclohexyl-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-2,5-dimethylhex-2-enamide.

32. The compound of claim 31, wherein P^1 is a monovalent radical of one of the following compounds:

a) (*S*,*E*)-4-((*S*)-2-((*S*)-3-(4-(Aminomethyl)phenyl)-3-methyl-2-(methylamino)butanamido)-*N*,3,3-trimethylbutanamido)-*N*-(benzylsulfonyl)-2,5-dimethylhex-2-enamide;

b) (*S*,*E*)-*N*-(4-(1-Aminocyclopropyl)benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

c) (*S*,*E*)-*N*-(4-(1-Aminocyclopropyl)phenylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide;

d) (*S*,*E*)-*N*-(4-(Aminomethyl)benzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide, or

e) (*S*,*E*)-*N*-(4-Aminobenzylsulfonyl)-2,5-dimethyl-4-((*S*)-*N*,3,3-trimethyl-2-((*S*)-3-methyl-2-(methylamino)-3-phenylbutanamido)butanamido)hex-2-enamide.

33. The compound of any one of claims 21 to 32, wherein L^1 comprises SPDP, SMCC, vcPABC, MCvcPABC, MTvc, ADvc, maleimide, NHS, biotin, streptavidin, NeutrAvidin, a glycoside, or a combination thereof.

34. The compound of any one of claims 21 to 32, wherein L^1 comprises MTvc, ADvc, maleimide or NHS.

35. A pharmaceutical composition comprising the compound of any one of claims 1 to 34, and a pharmaceutically acceptable carrier, diluent or excipient.

36. A method of treating a disease characterized by abnormal expression of placental-like chondroitin sulfate A (plCSA) in a mammal comprising administering to a mammal in need thereof an effective amount of the compound of any one of claims 1 to 34, or the pharmaceutical composition of claim 35.

37. Use of the compound of any one of claims 1 to 34 in the manufacture of a medicament for treating a disease characterized by abnormal expression of placental-like chondroitin sulfate A (plCSA) in a mammal.

38. The method of claim 36, or the use of claim 37, wherein the disease is cancer.

39. The method or use of claim 38, wherein the cancer is a carcinoma, a sarcoma, a hematopoietic cancer, or a tumor of neuroepithelial tissue.

40. The method of claim 36, or the use of claim 37, wherein the disease is arthritis, arthrosis, multiple sclerosis, neural damage, cartilage damage, or psoriasis.

Zymeworks Inc. and
VAR2 Pharmaceuticals APS

Patent Attorneys for the Applicant/Nominated Person

SPRUSON & FERGUSON

Figure 1

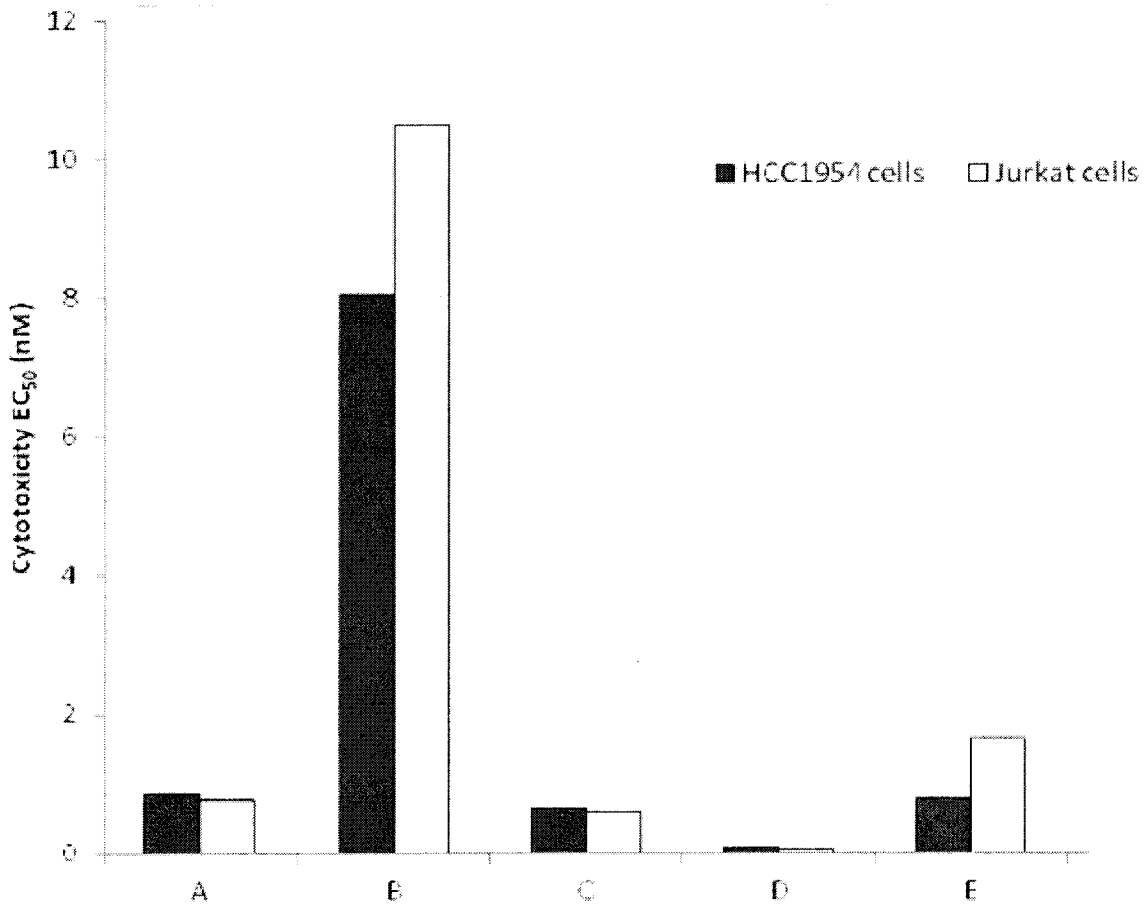

12
10
8
6
4
2
0
Cytotoxicity EC_{50} (nM)
HCC1954 cells
Jurkat cells
A
B
C
D
E

Figure 2

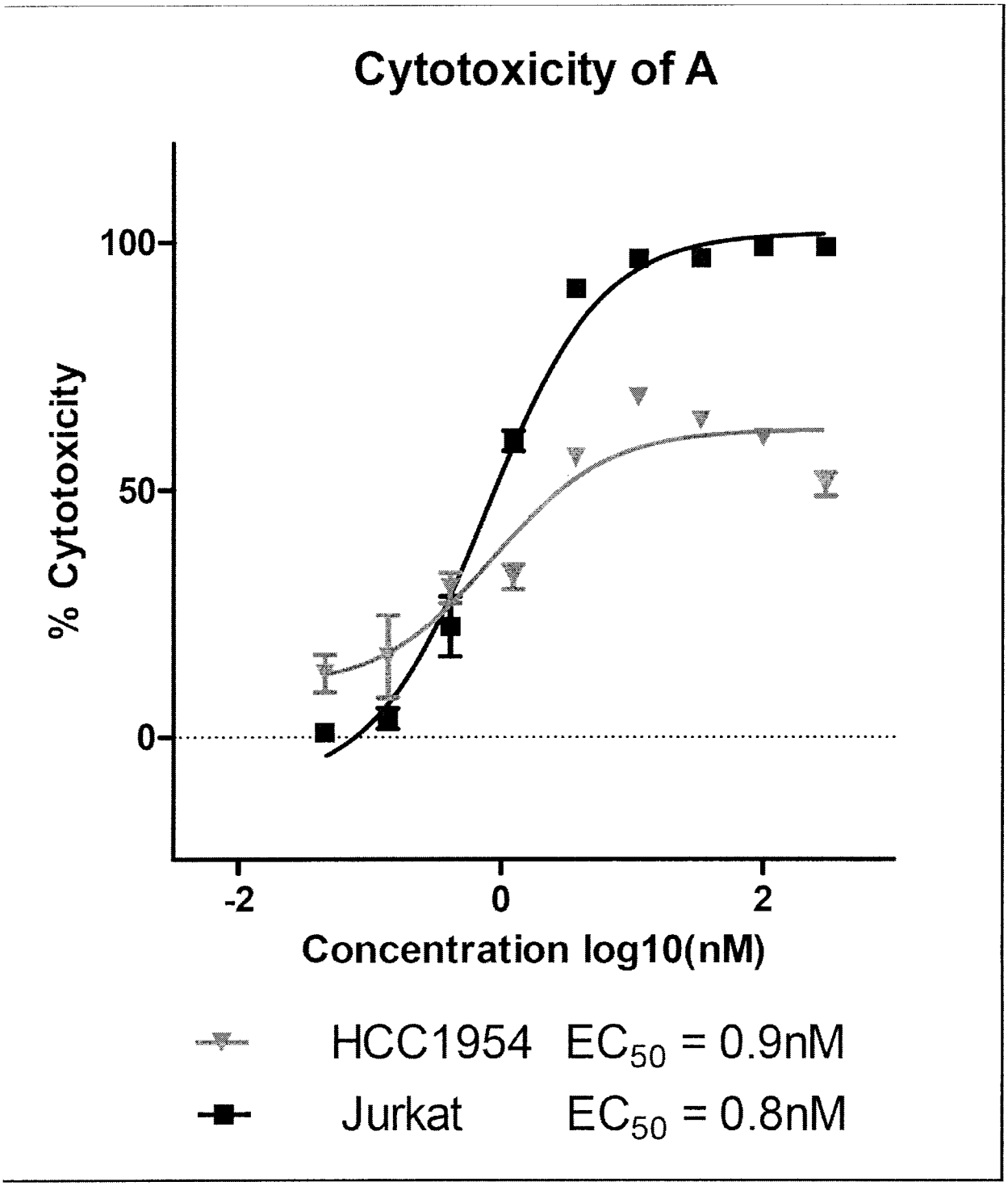

Cytotoxicity of A
% Cytotoxicity
100
50
0
-2
0
2
Concentration log10(nM)
HCC1954 EC50 = 0.9nM
Jurkat EC50 = 0.8nM

Figure 3

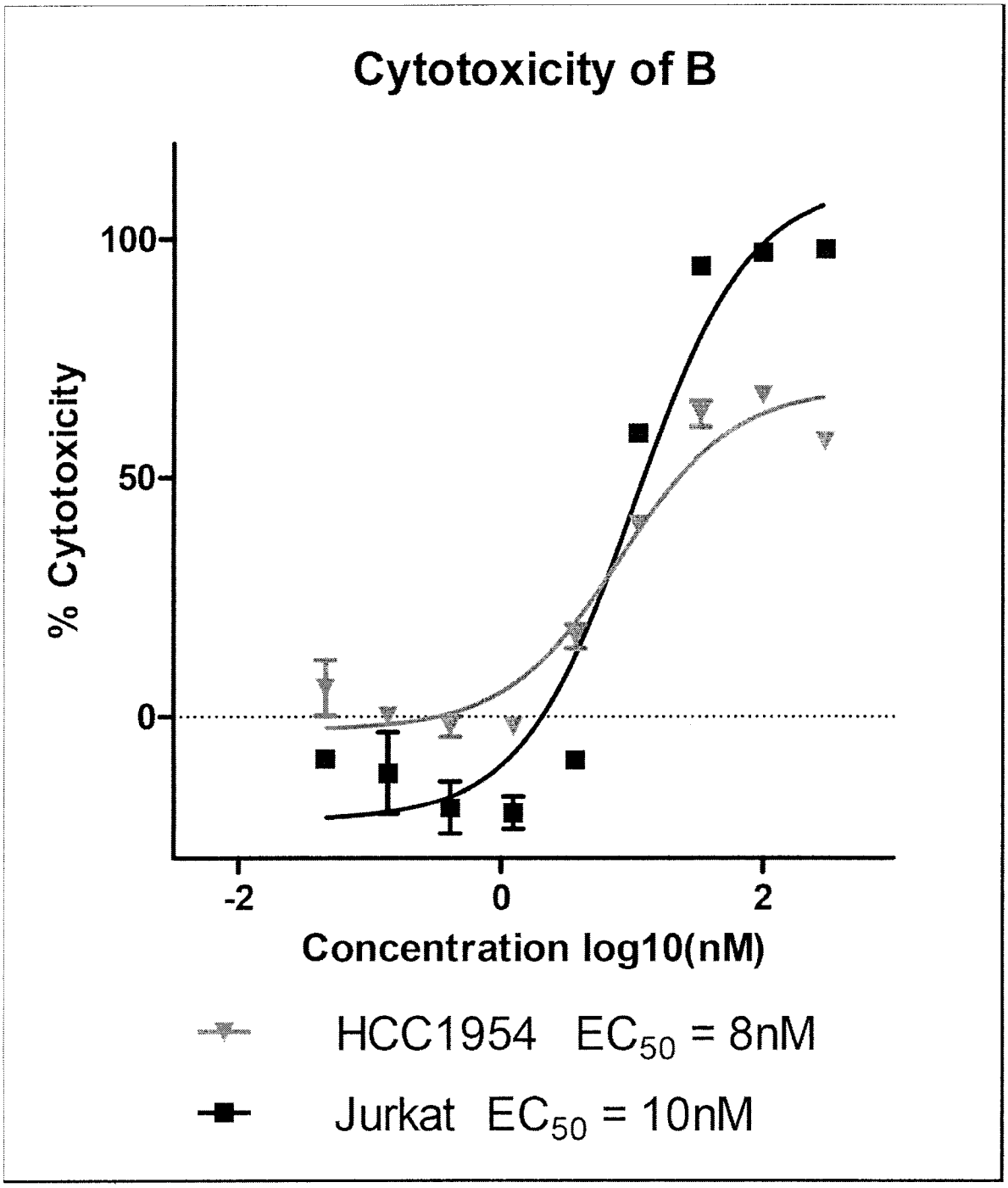

Cytotoxicity of B
100
50
0
% Cytotoxicity
-2
0
2
Concentration log10(nM)
HCC1954 EC50 = 8nM
Jurkat EC50 = 10nM

Figure 4

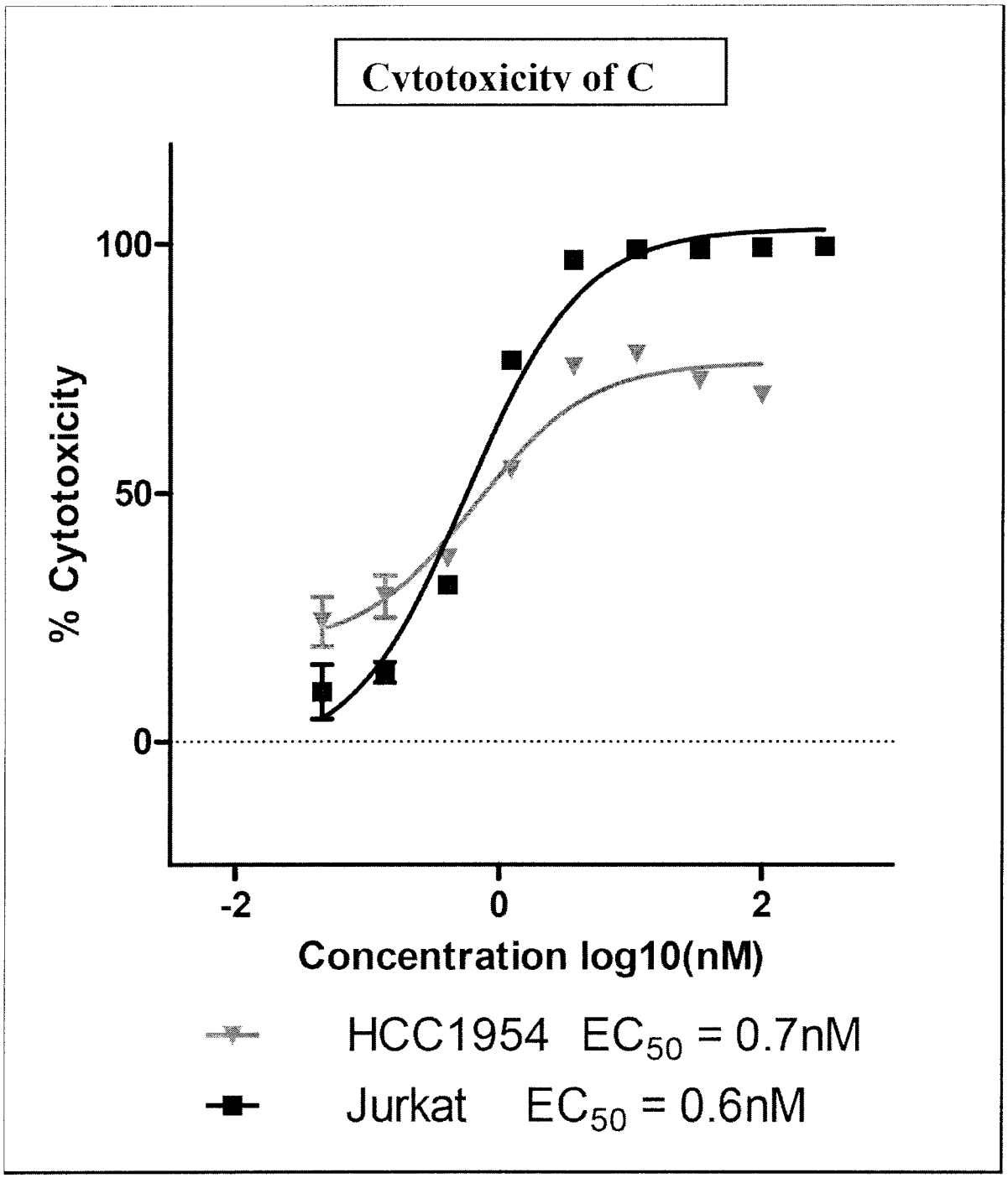

Cytotoxicity of C
100
50
0
% Cytotoxicity
-2
0
2
Concentration log10(nM)
HCC1954 EC50 = 0.7nM
Jurkat EC50 = 0.6nM

Figure 5

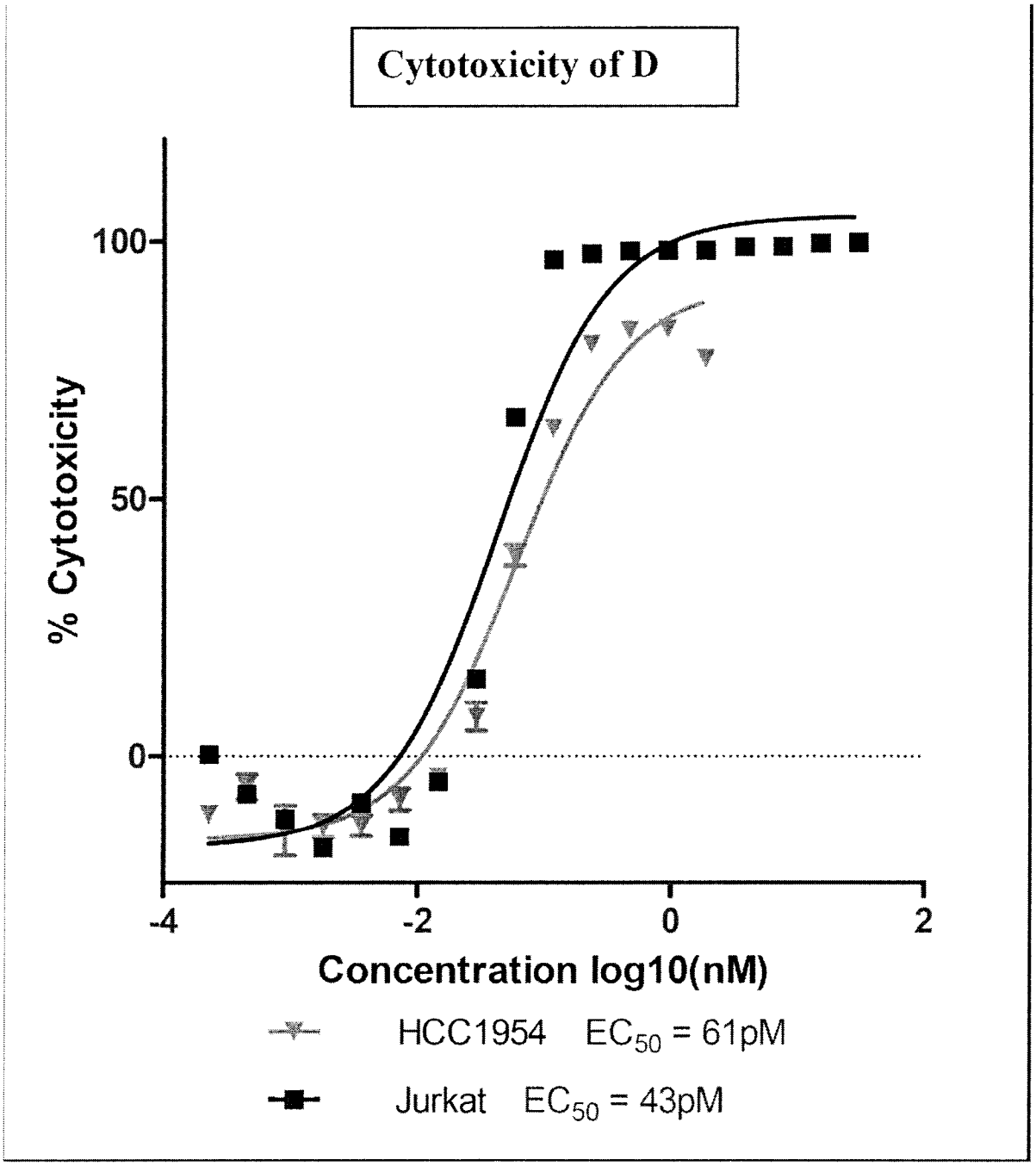

Cytotoxicity of D
100
50
0
% Cytotoxicity
-4
-2
0
2
Concentration log10(nM)
HCC1954 EC50 = 61pM
Jurkat EC50 = 43pM

Figure 6

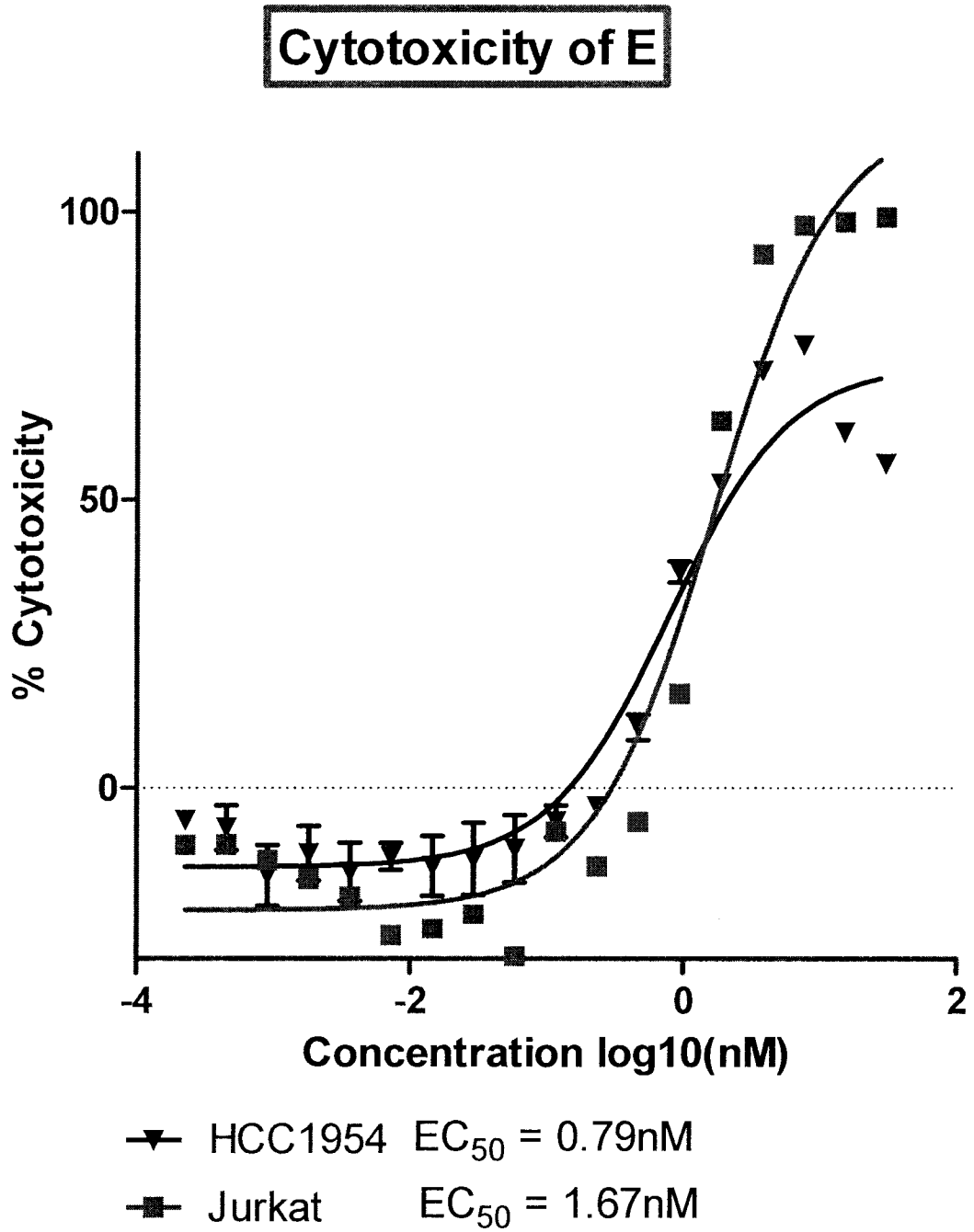

Cytotoxicity of E
100
50
0
% Cytotoxicity
-4
-2
0
2
Concentration log10(nM)
HCC1954 EC50 = 0.79nM
Jurkat EC50 = 1.67nM

Figure 7

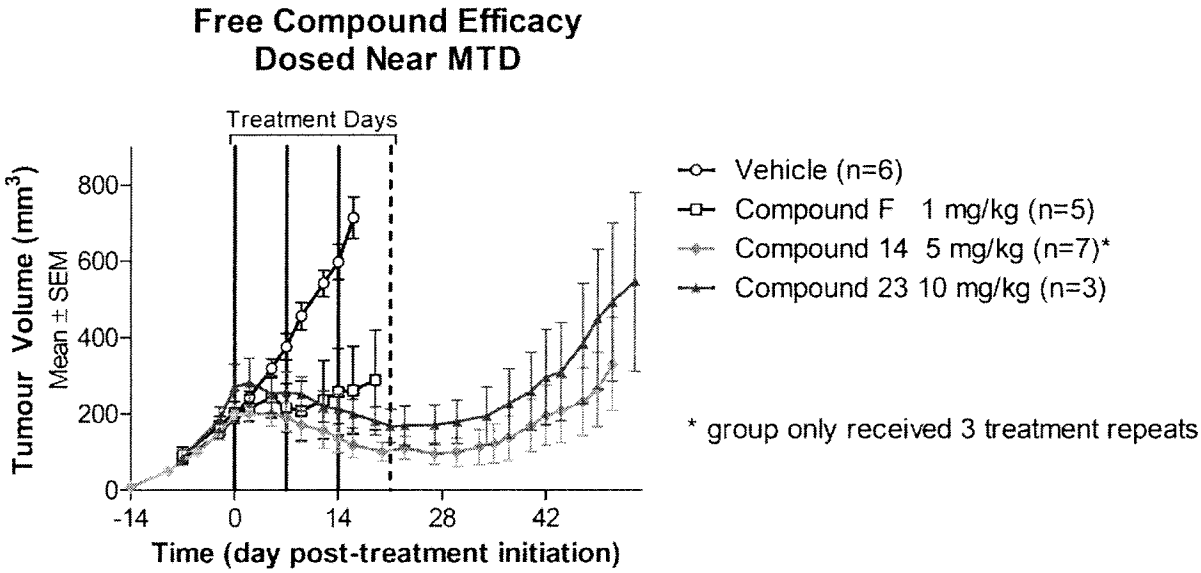

Free Compound Efficacy
Dosed Near MTD
Treatment Days
Tumour Volume (mm3)
Mean ± SEM
800
600
400
200
0
-14
0
14
28
42
Time (day post-treatment initiation)
Vehicle (n=6)
Compound F 1 mg/kg (n=5)
Compound 14 5 mg/kg (n=7)*
Compound 23 10 mg/kg (n=3)
* group only received 3 treatment repeats

Figure 8

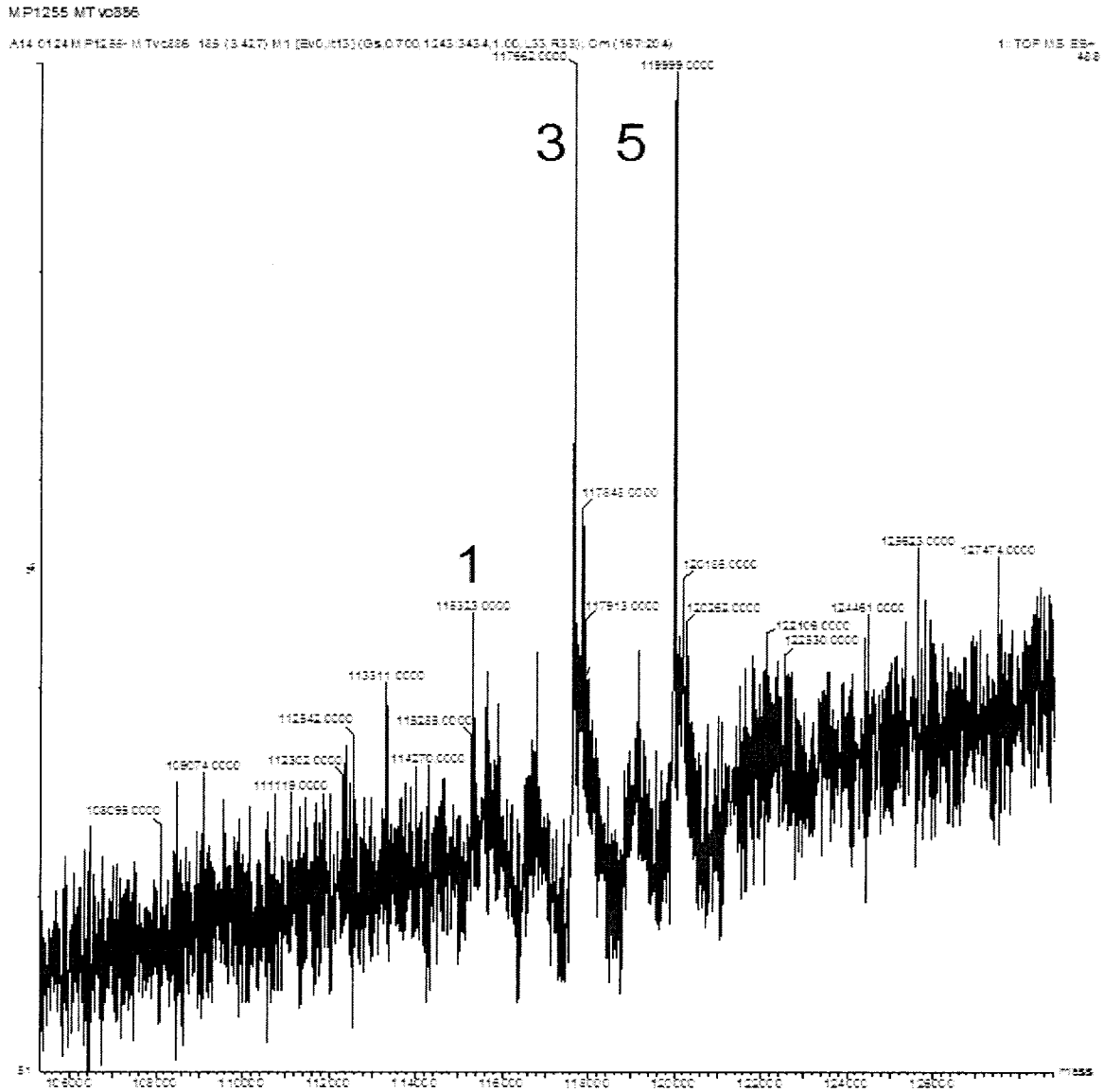

MP1255 MT vo856
3
5
1
117952.0000
119999.0000
117848.0000
116323.0000
117913.0000
120158.0000
120282.0000
123461.0000
122108.0000
122330.0000
125823.0000
127474.0000
113311.0000
112342.0000
115288.0000
109074.0000
112302.0000
114270.0000
111119.0000
108099.0000
m/z

Figure 9

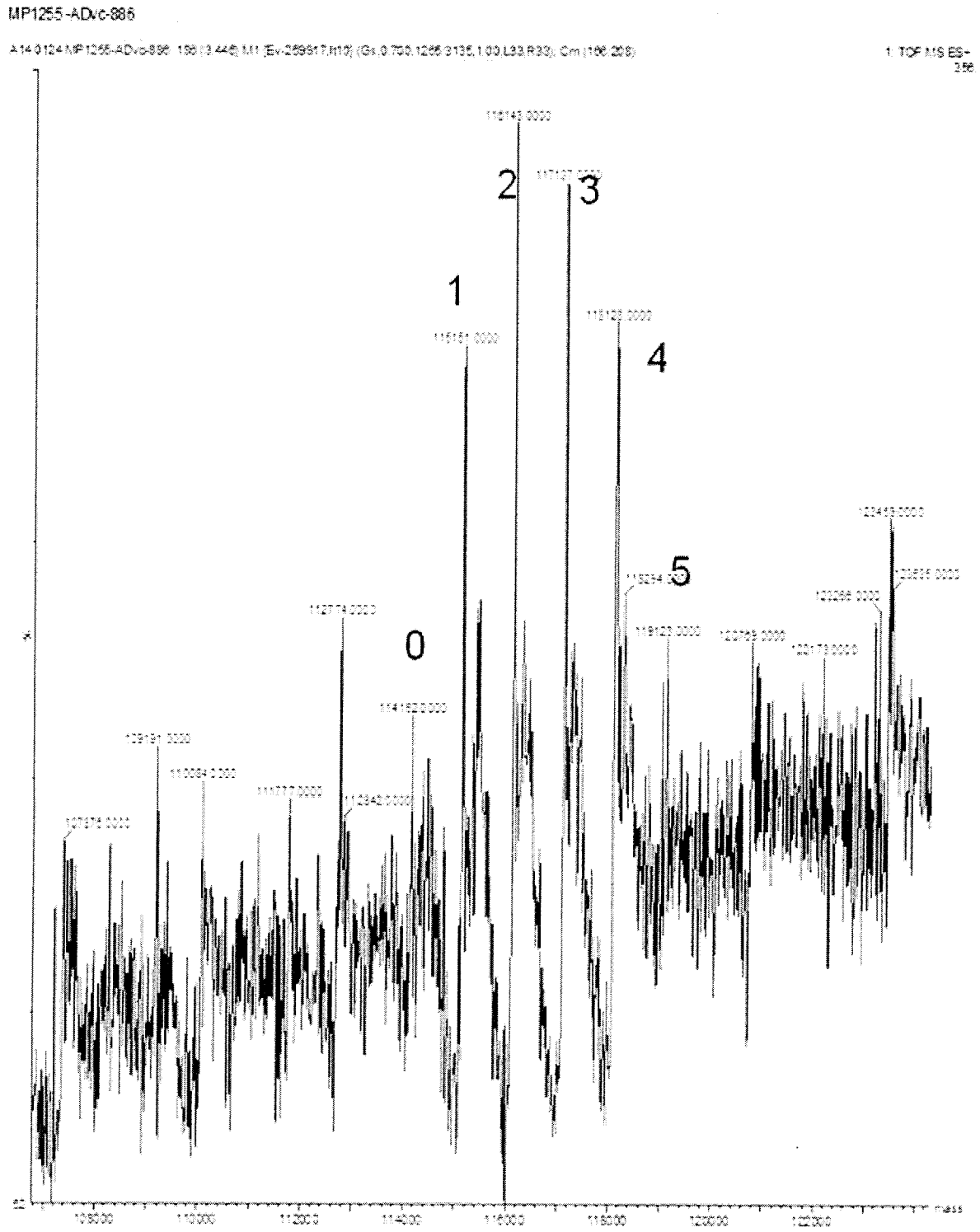


Figure 10

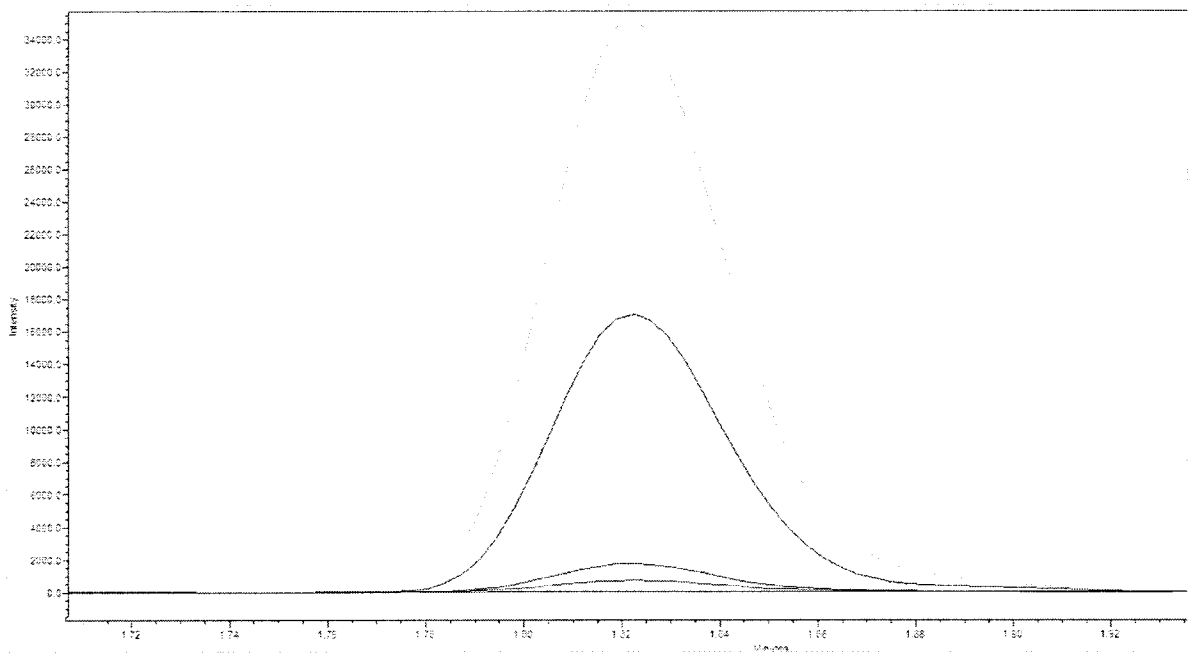


Figure 11

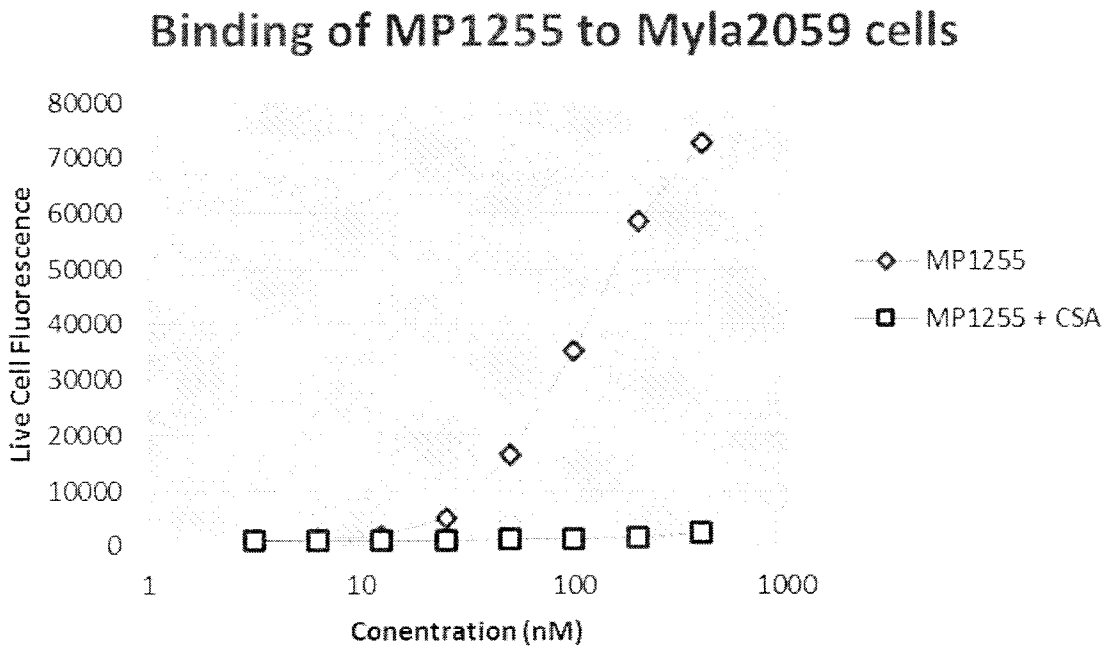

Binding of MP1255 to Myla2059 cells
Live Cell Fluorescence
80000
70000
60000
50000
40000
30000
20000
10000
0
1
10
100
1000
Conentration (nM)
MP1255
MP1255 + CSA

Figure 12

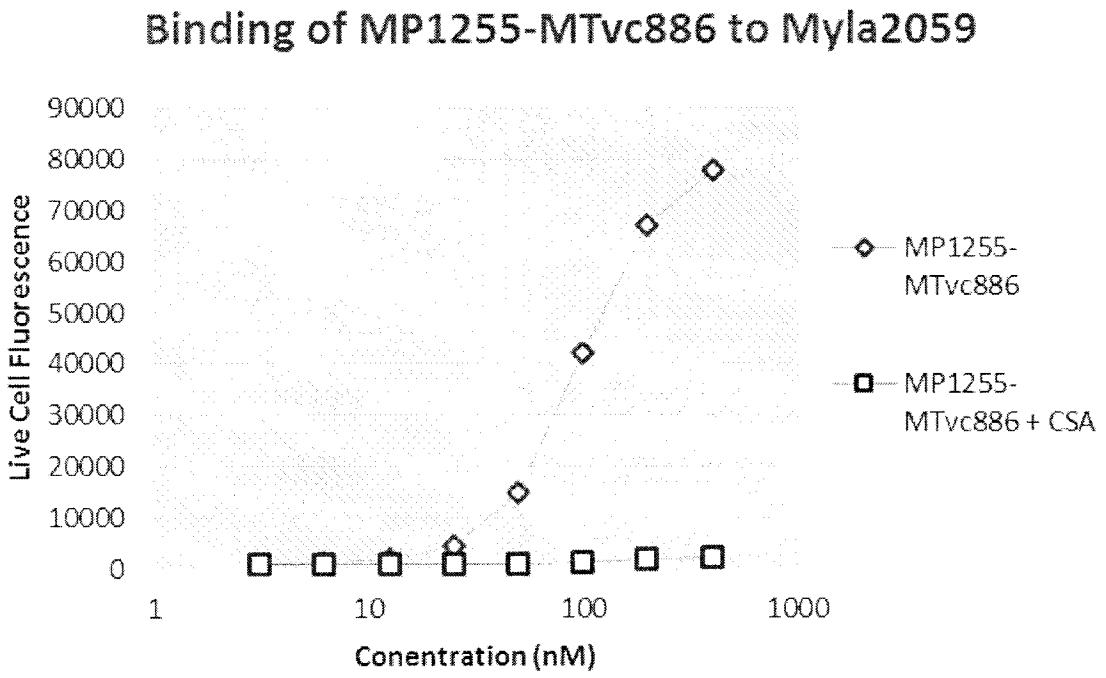

Binding of MP1255-MTvc886 to Myla2059
Live Cell Fluorescence
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
1
10
100
1000
Conentration (nM)
MP1255-MTvc886
MP1255-MTvc886 + CSA

Figure 13

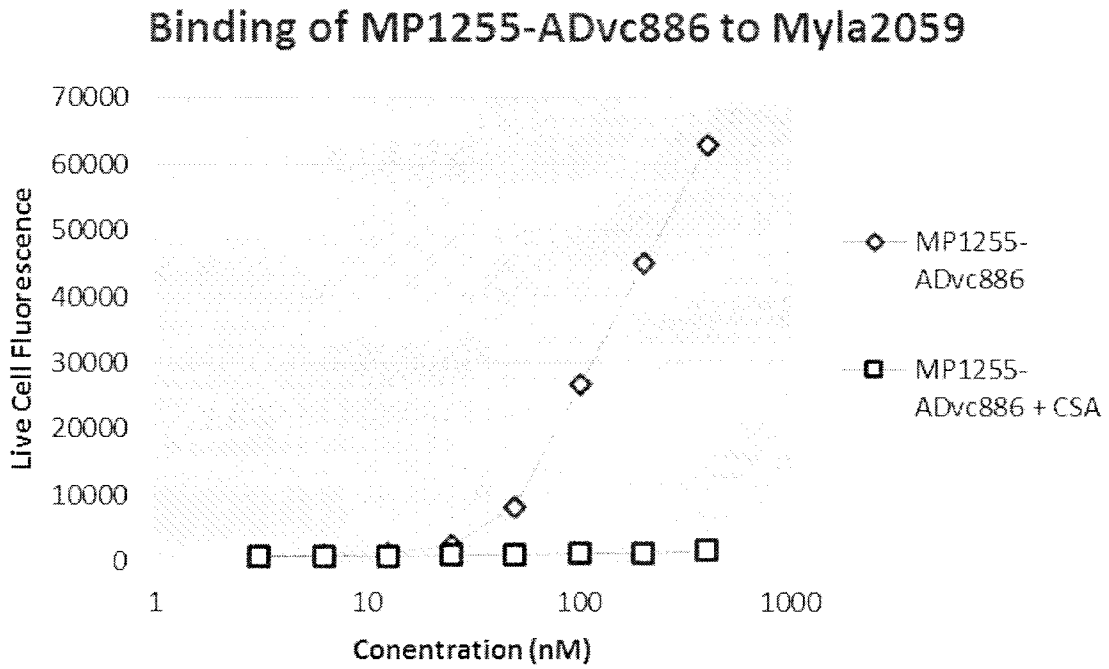

Binding of MP1255-ADvc886 to Myla2059
Live Cell Fluorescence
70000
60000
50000
40000
30000
20000
10000
0
1
10
100
1000
Conentration (nM)
MP1255-ADvc886
MP1255-ADvc886 + CSA

Figure 14

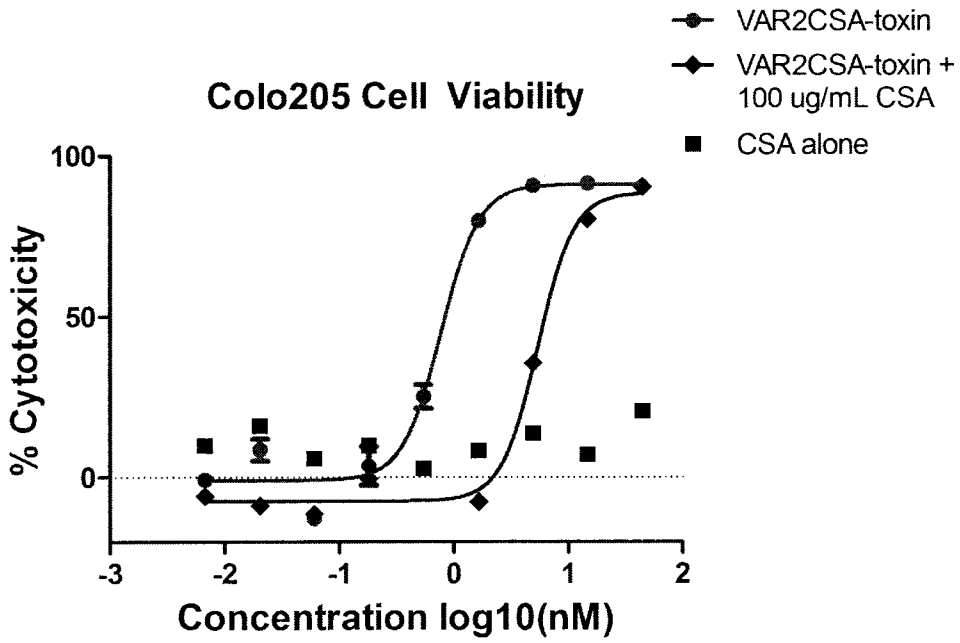

VAR2CSA-toxin
VAR2CSA-toxin + 100 ug/mL CSA
CSA alone
Colo205 Cell Viability
% Cytotoxicity
100
50
0
-3
-2
-1
0
1
2
Concentration log10(nM)

Figure 15

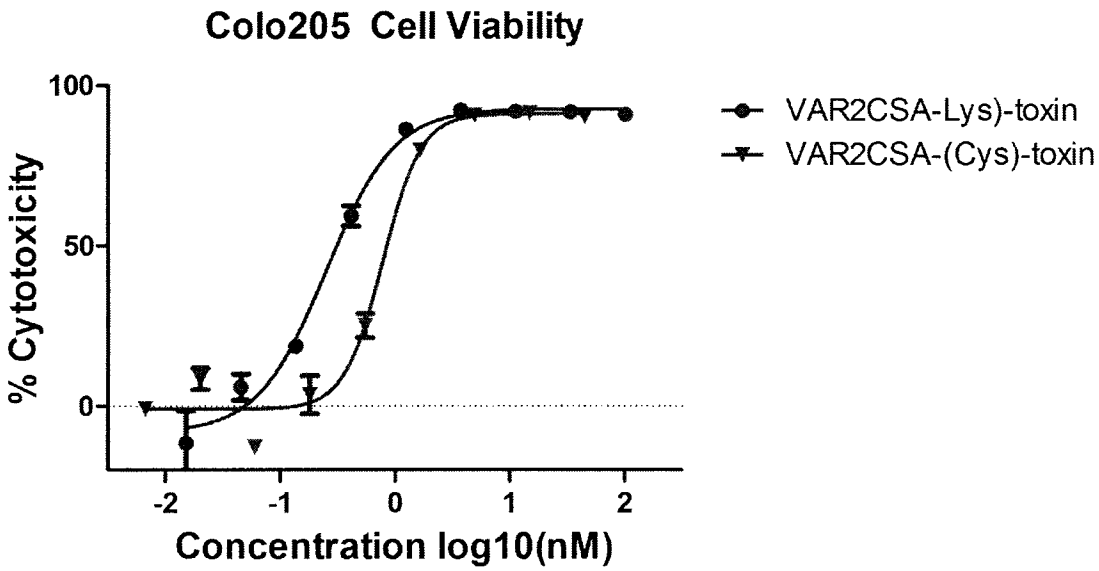

Colo205 Cell Viability
% Cytotoxicity
100
50
0
-2
-1
0
1
2
Concentration log10(nM)
VAR2CSA-Lys)-toxin
VAR2CSA-(Cys)-toxin

Figure 16

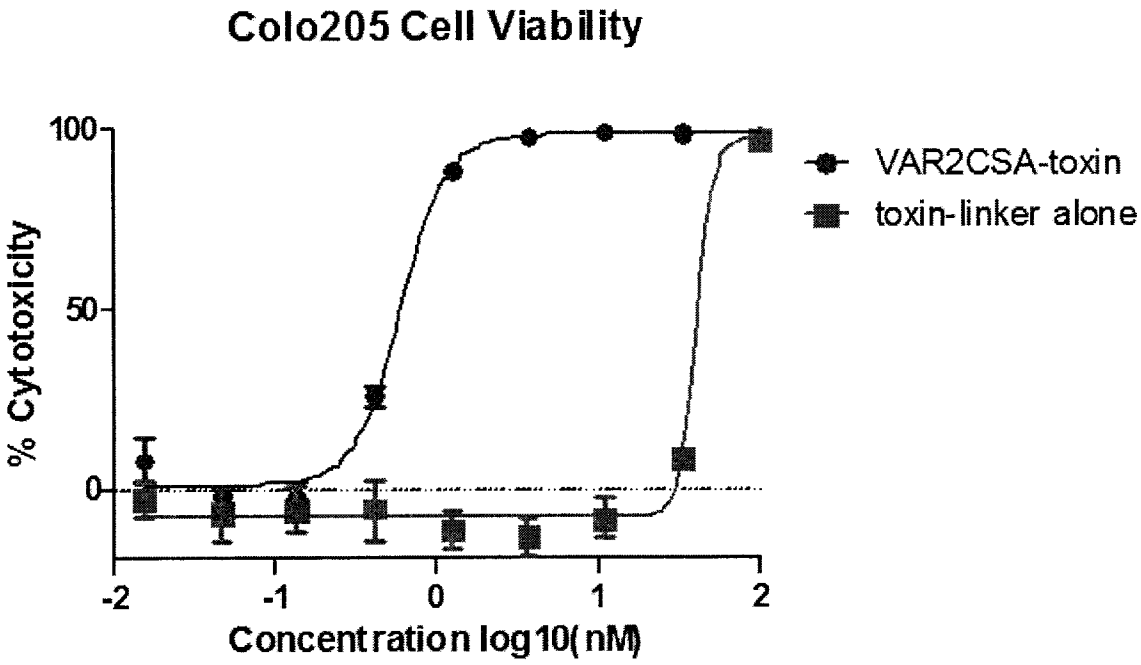

Colo205 Cell Viability
% Cytotoxicity
100
50
0
-2
-1
0
1
2
Concentration log10(nM)
VAR2CSA-toxin
toxin-linker alone

Figure 17

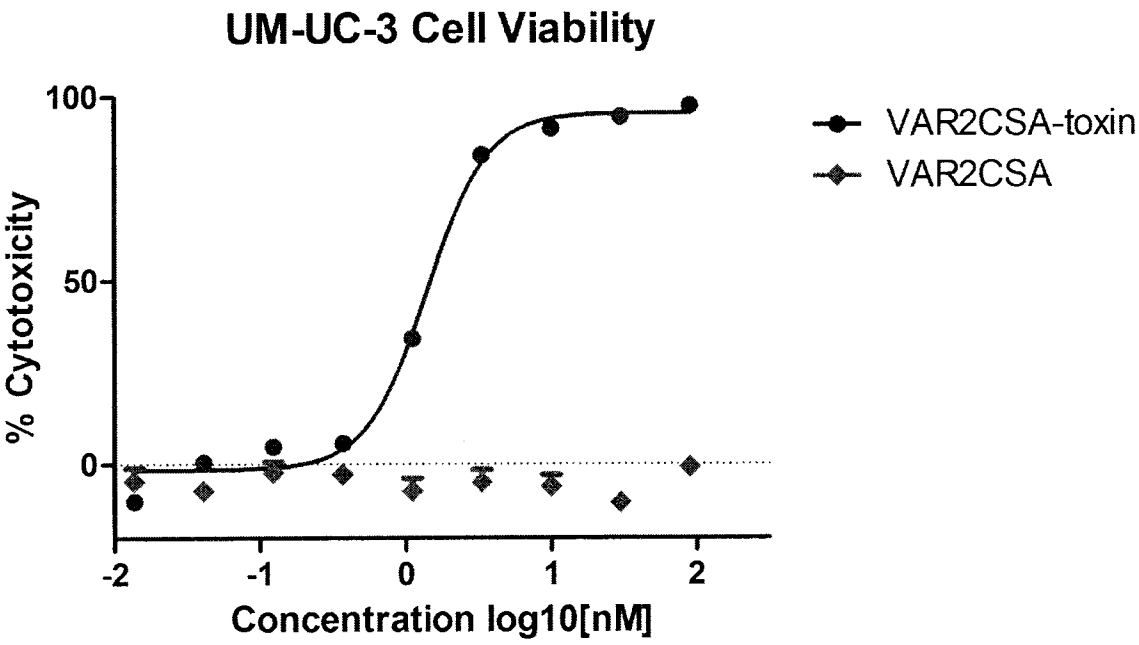

UM-UC-3 Cell Viability
% Cytotoxicity
100
50
0
-2
-1
0
1
2
Concentration log10[nM]
VAR2CSA-toxin
VAR2CSA

Figure 18

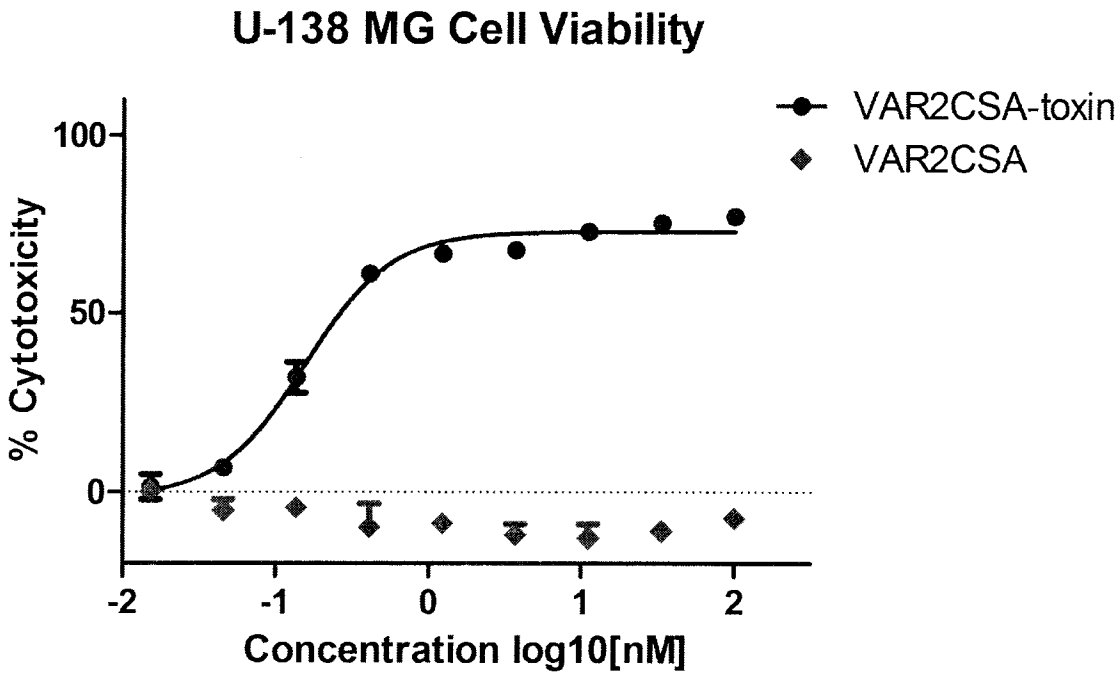

U-138 MG Cell Viability
VAR2CSA-toxin
VAR2CSA
% Cytotoxicity
100
50
0
-2
-1
0
1
2
Concentration log10[nM]

Figure 19

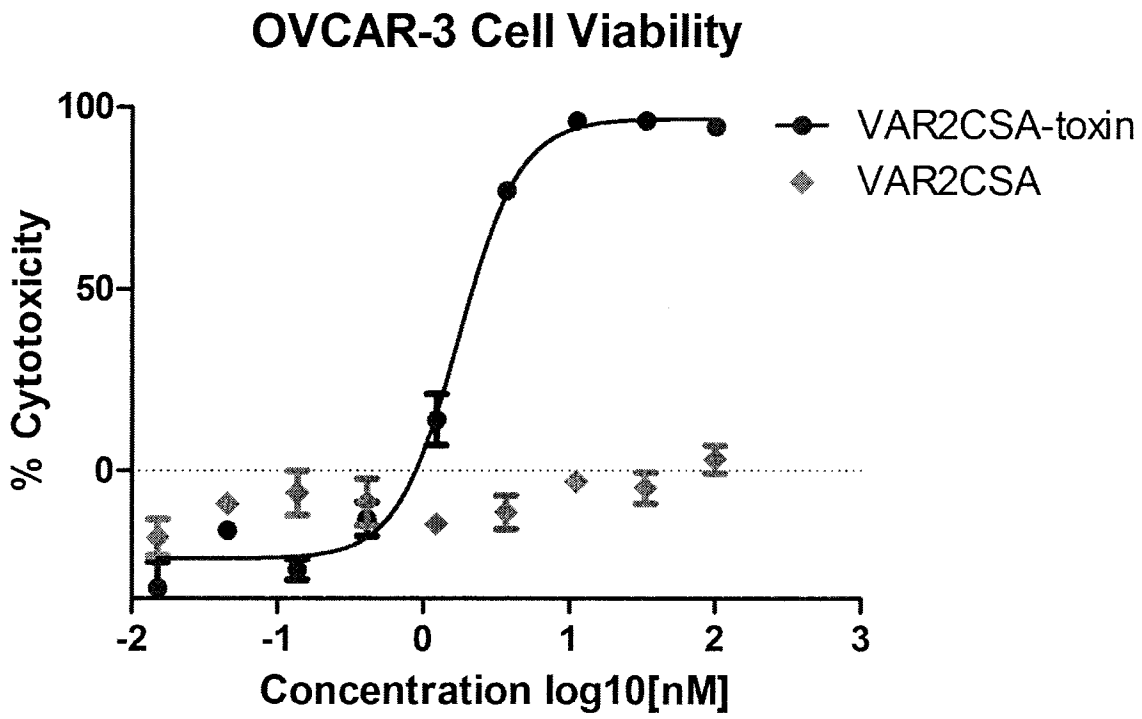

OVCAR-3 Cell Viability
% Cytotoxicity
100
50
0
-2
-1
0
1
2
3
Concentration log10[nM]
VAR2CSA-toxin
VAR2CSA

Figure 20

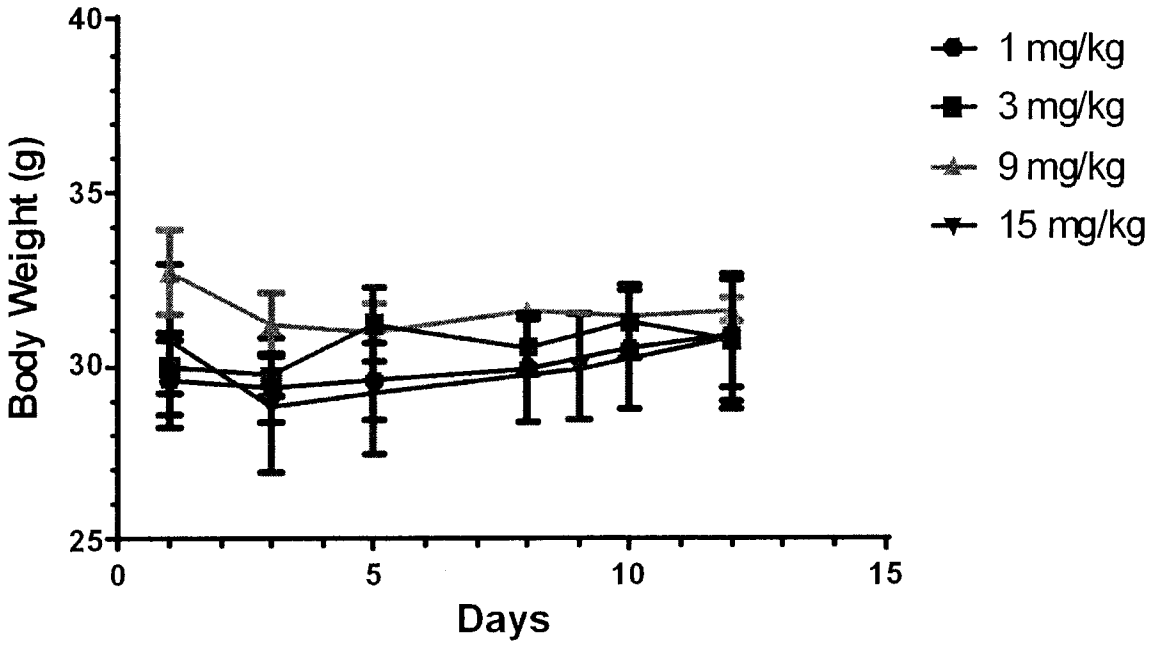

40
35
30
25
Body Weight (g)
0
5
10
15
Days
1 mg/kg
3 mg/kg
9 mg/kg
15 mg/kg

Figure 21

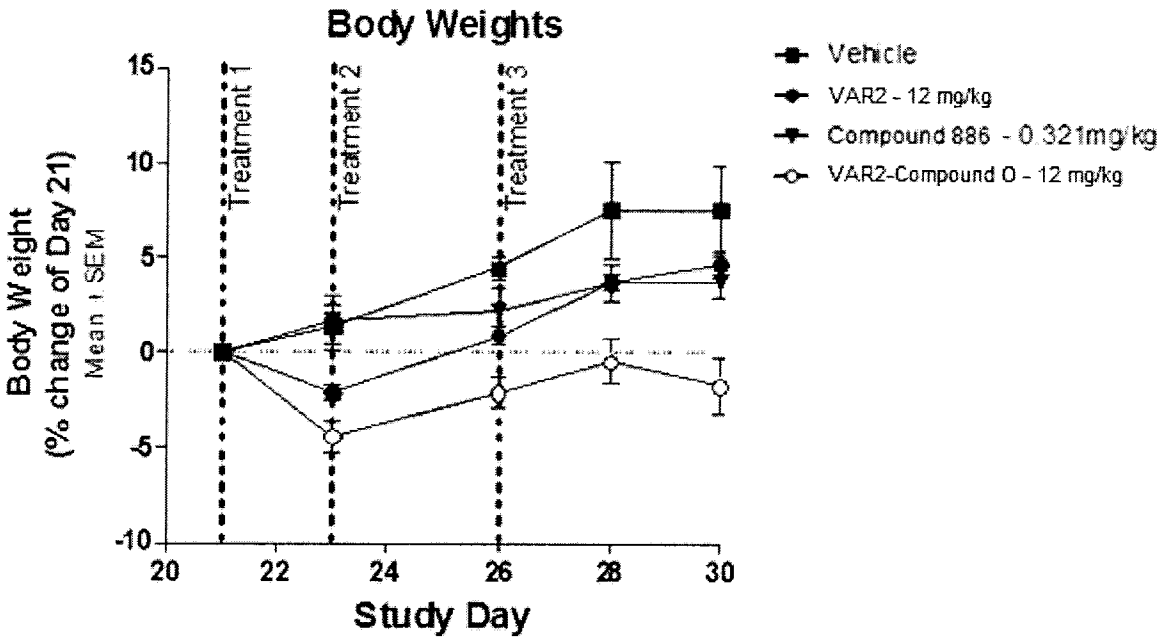

Body Weights
Body Weight
(% change of Day 21)
Mean ± SEM
Treatment 1
Treatment 2
Treatment 3
15
10
5
0
-5
-10
20
22
24
26
28
30
Study Day
Vehicle
VAR2 - 12 mg/kg
Compound 886 - 0.321mg/kg
VAR2-Compound O - 12 mg/kg

Figure 22

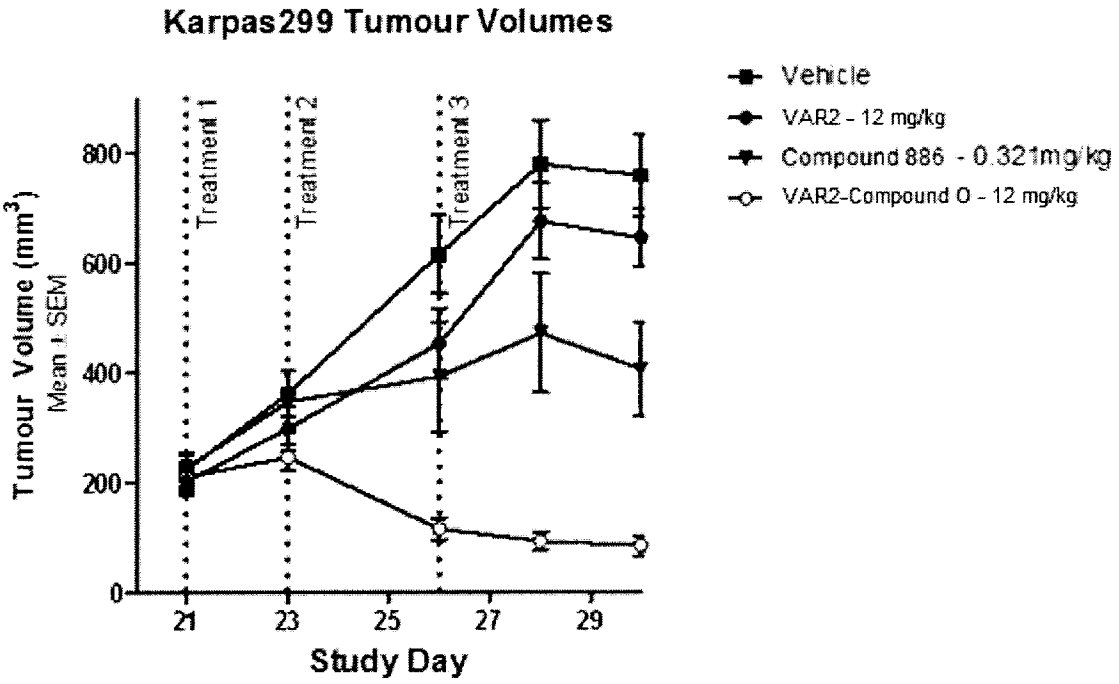

Karpas299 Tumour Volumes
Tumour Volume (mm3)
Mean ± SEM
Treatment 1
Treatment 2
Treatment 3
800
600
400
200
0
21
23
25
27
29
Study Day
Vehicle
VAR2 - 12 mg/kg
Compound 886 - 0.321mg/kg
VAR2-Compound O - 12 mg/kg

Figure 23

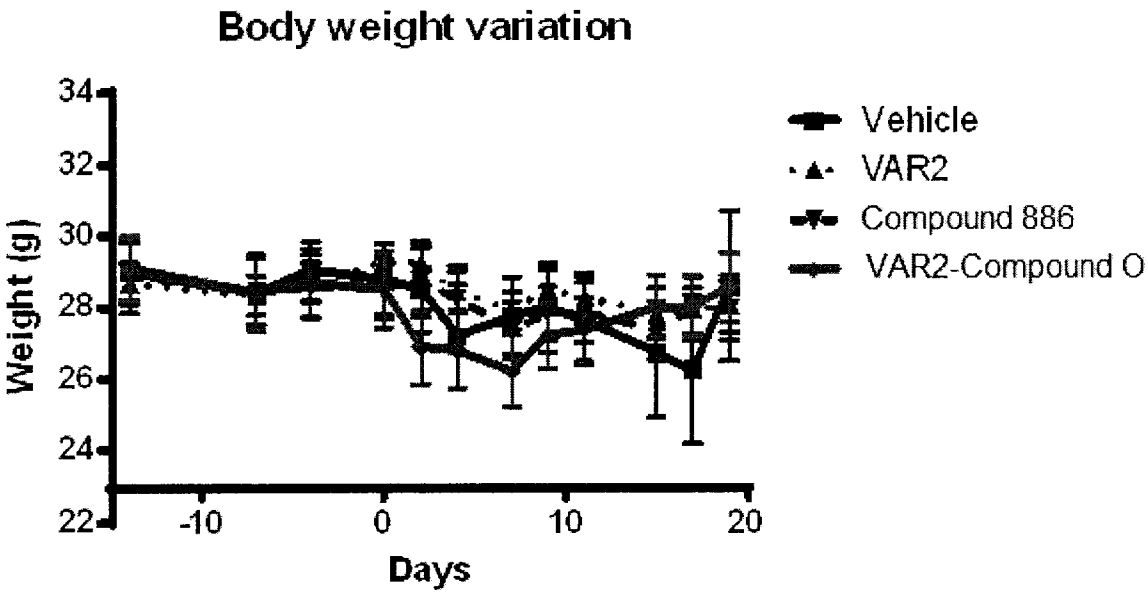
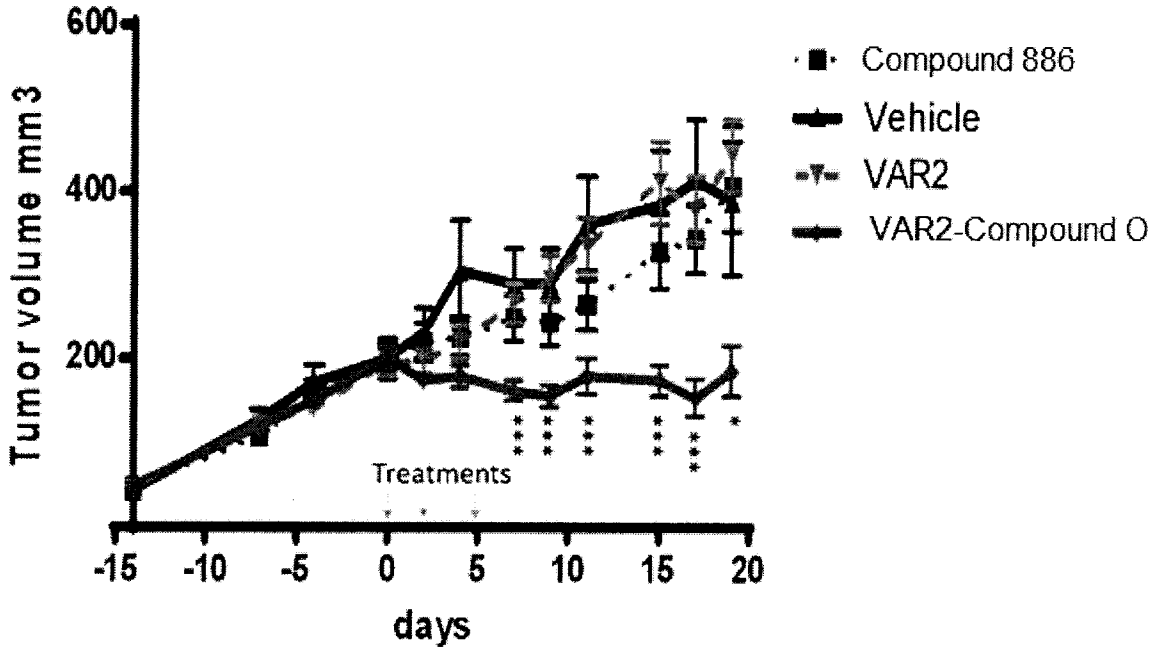

Body weight variation
Weight (g)
34
32
30
28
26
24
22
-10
0
10
20
Days
Vehicle
VAR2
Compound 886
VAR2-Compound O

Figure 24


600
400
200
Tumor volume mm3
Treatments
-15
-10
-5
0
5
10
15
20
days
Compound 886
Vehicle
VAR2
VAR2-Compound O

SEQUENCES

>fcr3 745 amino acids | 640 aa; underlined sequence corresponds to the ID1 domain of FCR3, Sequence in bold corresponds to DBL2Xb domain of FCR3. Remaining sequence is ID2a (SEQ ID NO:1)

NYIKGDPYFAEYATKLSFILNPSDANNPSGETANHNDEACNCNESGISSVGQAQTSGPSSNKT
CITHSSIKTNKKKECKDVKLGVRENDKDLKICVIEDTSLSGVDNCCCQDLLGILQENCSDNKR
GSSSNDSCDNKNQDECQKKLEKVFAS**LTNGYKCDKCKSGTSRSKKKWIWKKSSGNEEGL**
QEEYANTIGLPPRTQSLYLGNLPKLENVCEDVKDINFDTKEKFLAGCLIVSFHEGKNLK
KRYPQNKNSGNKENLCKALEYSFADYGDLIKGTSIWDNEYTKDLELNLQNNFGKLFGK
YIKKNNTAEQDTSYSSLDELRESWWNTNKKYIWTAMKHGAEMNITTCNADGSVTGSGS
SCDDIPTIDLIPQYLRFLQEWVENFCEQRQAKVKDVITNCKSCKESGNKCKTECKTKCK
DECEKYKKFIEACGTAGGGIGTAGSPWSKRWDQIYKRYSKHIEDAKRNRKAGTKNCGT
SSTTNAAASTDENKCVQSDIDSFFKHLIDIGLTTPSSYLSNVLDDNICGADKAPWTTYTTY
TTTEKCNKERDKSKSQSSDTLVVVNVPSPLGNTPYRYKYACQCKIPTNEETCDDRKEYMN
QWSCGSARTMKRGYKNDNYELCKYNGVDVKPTTVRSNSSKLD

>gi | 254952610 | gb | ACT97135.1 | VAR2CSA [Plasmodium falciparum] | 341 aa (SEQ ID NO:2)

KCDKCKSGTSRSRKIWTWRKSSGNKEGLQEEYANTIGLSPRTQLLYLGNLRKLENVCEDVTD
INFDTKEKFLAGCLIAAFHEGKNLKKRYLEKKKGDNNSKLCKDLKYSFADYGDLIKGTSIWD
NDFTKDLELNLQQIFGKLFRKYIKKKNISTEQDTSYSSLDELRESWWNTNKKYIWLAMKHGA
GMNSTTCSCSGDSSSGENQTNSCDDIPTIDLIPQYLRFLQEWVEHFCEQRQAKVKDVITNCNS
CKESGGTCNSDCEKKCKNKCDAYKTFIEDCKGVGGTGTAGSSWVKRWYQIYMRYSKYIED
AKRNRKAGTKSCGTSSTTNVSVSTDENKCVQS-

>M24 745 amino acids | 656 aa (SEQ ID NO:3)

DYIKGDPYFAEYATKLSFILNSSDANNPSGETANHNDEVCNPNESEISSVGQAQTSDPSSN
KTCNTHSSIKANKKKVCKHVKLGINNNDKVLRVCVIEDTSLSGVENCCFKDLLGILQEN
CSDNKSGSSSNGSCNNKNQEACEKNLEKVLASLTNCYKCDKCKSGTSTVNKNWIWKKS
SGNKEGLQKEYANTIGLPPRTHSLYLGNLPKLENVCEDVKDINFDTKEKFLAGCLIAAF
HEGKNLKKRYPQNKNDDNNSKLCKALEYSFADYGDLIKGTSIWDNEYTKDLELNLQQI
FGKLFRKYIKKNISTEQDTLYSSLDELRESWWNTNKKYIWLAMKHGAGMNITTCCGDG
SVTGSGSSCDDIPTIDLIPQYLRFLQEWVEHFCKQRQEKVKDVINSCNSCKNTSSKTKLG
DTCNSDCEKKCKIECEKYKKFIEECRTAVGGTAGSSWSKRWDQIYKMYSKHIEDAKRN
RKAGTKNCGITTGTISGESSGANSGVTTTENKCVQSDIDSFFKHLIDIGLTTPSSYLSIVLD

DNICGDDKAPWTTYTTYTTYTTTEKCNKERDKSKSQQSNTSVVVNVPSPLGNTPHGYK
YACQCKIPTNEETCDDRKEYMNQWISDTSKNPKGSGSTNNDYELYTYNGVKETKLPKKLNS
PKLD

>KMWII 745 amino acids | 643 aa (SEQ ID NO:4)

DYIKDDPYSKEYTTKLSFILNSSDANTSSGETANHNDEACNCNESEISSVGQAQTSGPSSN
KTCITHSFIKANKKKVCKDVKLGVRENDKVLRVCVIEDTSLSGVDNCCCQDLLGILQEN
CSDNKRGSSSNGSCNNKNQDECQKKLEKVFVSLTNGYKCDKCKSGTSTVNKKWIWKK
SSGNEKGLQKEYANTIGLPPRTQSLYLGNLPKLGNVCEDVTDINFDTKEKFLAGCLIAAF
HEGKNLKISHEKKKGDNGKKLCKALEYSFADYGDLIKGTSIWDNEYTKDLELNLQKAF
GKLFGKYIKKNIASDENTSYSSLDELRESWWNTNKKYIWTAMKHGAEMNSTMCNADG
SVTGSGSSCDDIPTTDFIPQYLRFLQEWVEHFCKQRQEKVNAVIENCNSCKNTSGERKIG
GTCNGDCKTECKNKCEAYKNFIEDCKGGDGTAGSSWVKRWDQIYKRYSKHIEDAKRN
RKAGTKSCGPSSITNASVSTDENKCVQSDIDSFFKHLIDIGLTTPSSYLSIVLDENNCGEDN
APWTTYTTYTTTEKCNKDKKKSKSQSCNTAVVVNVPSPLGNTPHEYKYACQCKIPTTEE
TCDDRKEYMNQWISDTSKKQKGSGSTNNDYELYTYTGVKETKLPKKLNSPKLD

>1248 745 amino acids | 640 aa (SEQ ID NO:5)

SYVKNDPYSKEYVTKLSFILNPSDANNPSGETANHNDEACNPNESEIASVGQAQTSDRLS
QKACITHSFIGANKKIVCKDVKLGVREKDKDLKICVIEDDSLRGVENCCFKDLLGILQE
NCSDNKSGSSSNGSCNNKNQDECQKKLDEALASLHNGYKCDKCKSGTSRSKKIWTWRK
FPGNGEGLQKEYANTIGLPPRTQSLYLGNLRKLENVCKGVTDINFDTKEKFLAGCLIAA
FHEGKNLKISNKKKNDDNGKKLCKDLKYSFADYGDLIKGTSIWDNEYTKDLELNLQKI
FGKLFRKYIKKNIASDENTLYSSLDELRESWWNTNKKYIWLAMKHGTTCSSGSGDNGD
GSVTGSGSSCDDMSTIDLIPQYLRFLQEWVEHFCKQRQEKVKDVIENCKSCKNTSGERII
GGTCGSDCKTKCKGECDAYKNFIEECKRGDGTAGSPWSKRWDQIYMRYSKYIEDAKR
NRKAGTKNCGTSSTTNAAENKCVQSDIDSFFKHLIDIGLTTPSSYLSIVLDENICGDDKAP
WTTYTTYTTTEKCNKETDKSKSQSCNTAVVVNVPSPLGNTPHGYKYACECKIPTTEETCD
DRKEYMNQWISDTSKKPKGGRSTNNDYELYTYNGVKETKLPKKSSSSKLD

>gi | 254952618 | gb | ACT97139.1 | VAR2CSA [Plasmodium falciparum] | 358 aa (SEQ ID NO:6)

KCEKCKSEQSKKNNNIWIWRKFPGNGEGLQKEYANTIGLPPRTHSLYLGNLPKLENVCKDVK
DINFDTKEKFLAGCLIAAFHEGKNLKTTYPQNKNADNNSKLCKDLKYSFADYGDLIKGTSIW
DNDFTKDLELNLQKIFGKLFRKYIKKNIASDENTLYSSLDELRESWWNTNKKYIWLAMKHG
AEMNSTMCNGDGSVTGSSDSGSTTCSGDNGSISCDDIPTIDLIPQYLRFLQEWVEHFCKQRQE

KVKPVIENCKSCKNTSGERIIGGTCGSDCEKKCKGECDAYKKFIEECKGGGGGTGTAGSPWS
KRWDQIYKRYSKYIEDAKRNRKAGTKSCGPSSTTNAAASTTESKCVQS

>gi | 254952592 | gb | ACT97126.1 | VAR2CSA [Plasmodium falciparum] | 333 aa (SEQ ID NO:7)

KCDKCKSEQSKKNNKNWIWKQFPGNGEGLQKEYANTIGLPPRTHSLYLGNLPKLENVCKGV
TDINFDTKEKFLAGCLIAAFHEGKNLKTSHEKKKGDNGKKLCKDLKYSFADYGDLIKGTSIW
DNDFTKDLELNLQQIFGKLFRKYIKKNISAEQDTSYSSLDELRESWWNTNKKYIWLAMKHGT
TCSSGSGDNGDGSVTGSGSSCDDMPTTDFIPQYLRFLQEWVEHFCKQRQEKVNAVITNCKSC
KESGGTCNSDCEKKCKDECEKYKKFIEECRTAADGTAGSSWSKRWDQIYKMYSKHIEDAKR
NRKAGTKNCGTSSTTNAAENKCVQS

>gi | 90193467 | gb | ABD92329.1 | erythrocyte membrane protein 1 [Plasmodium falciparum] | 269 aa (SEQ ID NO:8)

DYIKDDPYSKEYTTKLSFILNSSDANTSSGETANHNDEACNCNESEIASVEQASISDRSSQKAY
ITHSSIKTNKKKVCKYVKLGINNNDKVLRVCVIEDTSLSGVENCCFKDLLGILQENCSDNKRG
SSFNDSCNNNNEEACQKKLEKVLASLTNGYKCEKCKSGTSRSKKKWIWKKSSGKEGGLQKE
YANTIGLPPRTQSLYLGNLPKLENVCKGVTDINFDTKEKFLAGCLIAAFHEGKNLKPSHQNKN
DDNNSKLCKDLKYSFADY

>gi | 254952616 | gb | ACT97138.1 | VAR2CSA [Plasmodium falciparum] | 333 aa (SEQ ID NO:9)

KCDKCKSGTSRSKKKWTWRKSSGNKEGLQKEYANTIGLPPRTHSLYLGNLRKLENVCEDVT
DINFDTKEKFLAGCLIAAFHEGKNLKTTYPQNKNDDNNSKLCKALKYSFADYGDLIKGTSIW
DNDFTKDLELNLQKIFGKLFRKYIKKNISTEQHTSYSSLDELRESWWNTNKKYIWLAMKHGA
EMNGTTCSCSGDSSDDIPTIDLIPQYLRFLQEWVEHFCKQRQAKVNAVINSCNSCKNTSGERK
LGGTCGSECKTECKNKCDAYKEFIDGTGSGGGTGTAGSSWVKRWDQIYKRYSKYIEDAKRN
RKAGSKNCGTSSTTNAAESKCVQS

>hb31 745 amino acids | 650 aa (SEQ ID NO:10)

SYVKNNPYSAEYVTKLSFILNSSDANTSSETPSKYYDEVCNCNESEISSVGQAQTSGPSSN
KTCITHSSIKTNKKKVCKDVKLGINNNDKVLRVCVIEDTSLSGVDNCCCQDLLGILQEN
CSDKNQSGSSSNGSCNNKNQDECQKKLEKVFASLTNGYKCDKCKSGTSRSKKKWIWR
KSSGNEEGLQKEYANTIGLPPRTQSLYLGNLRKLENVCKGVTDINFDTKEKFLAGCLIA
AFHEGKNLKTTYPQNKKKLCKDLKYSFADYGDLIKGTSIWDNEYTKDLELNLQKAFGK
LFRKYIKKNISTEQHTLYSSLDELRESWWNTNKKYIWLAMKHGAGMNSTTCCGDGSVT

GSGSSCDDIPTIDLIPQYLRFLQEWVEHFCKQRQEKVNAVIENCNSCKECGDTCNGECK
TECEKKCKIECEKYKTFIEECVTAVGGTSGSPWSKRWDQIYKRYSKYIEDAKRNRKAG
TKNCGITTGTISGESSGANSGVTTTENKCVQSDIDSFFKHLIDIGLTTPSSYLSIVLDDNIC
GADNAPWTTYTTYTTYTTTKNCDIKKKTPKSQPINTSVVVNVPSPLGNTPHGYKYACQC
KIPTTEESCDDRKEYMNQWIIDTSKKQKGSGSTNNDYELYTYNGVKETKLPKKSSSSKLD

>hb32 745 amino acids | 643 aa (SEQ ID NO:11)

SYVKDDPYSAEYVTKLSFILNSSDANTSSETPSKYYDEVCNCNESEISSVGQAQTSGPSSN
KTCITHSSIKTNKKKVCKDVKLGINNNDKVLRVCVIEDTSLSGVDNCCCQDLLGILQEN
CSDKNQSGSSSNGSCNNKNQDECQKKLEKVFASLTNGYKCDKCKSGTSRSKKKWIWR
KSSGNEEGLQKEYANTIGLPPRTQSLYLGNLPKLENVCKGVTDIIYDTKEKFLSGCLIAA
FHEGKNLKTSHEKKNDDNGKKLCKALEYSFADYGDLIKGTSIWDNDFTKDLELNLQKI
FGKLFRKYIKKNNTAEQDTSYSSLDELRESWWNTNKKYIWTAMKHGAGMNSTTCSGD
GSVTGSGSSCDDMPTIDLIPQYLRFLQEWVEHFCKQRQEKVKDVITNCNSCKECGDTCN
GECKTECKTKCKGECEKYKNFIEECNGTADGGTSGSSWSKRWDQIYKRYSKYIEDAKR
NRKAGTKNCGTSSTTNAAASTTENKCVQSDIDSFFKHLIDIGLTTPSSYLSNVLDDNICGE
DKAPWTTYTTYTTKNCDIQKKTPKPQSCDTLVVVNVPSPLGNTPHGYKYVCECKIPTTE
ETCDDRKEYMNQWIIDTSKKQKGSGSTNNDYELYTYNGVQIKQAAGTLKNSKLD

>gi | 90193475 | gb | ABD92333.1 | erythrocyte membrane protein 1 [Plasmodium falciparum] | 269 aa (SEQ ID NO:12)

NYIKGDPYSAEYATKLSFILNSSDTENASEKIQKNNDEVCNCNESEIASVEQAPISDRSSQKACI
THSSIKANKKKVCKHVKLGVRENDKDLKICVIEDTSLSGVDNCCCQDLLGILQENCSDNKSG
SSSNGSCNNNNEEICQKKLEKVLASLTNGYKCDKCKSGTSTVNKNWIWKKYSGKEGGLQEE
YANTIGLPPRTQSLYLGNLPKLENVCEDVKDINFDTKEKFLAGCLIAAFHEGKNLKTSNKKK
NDDNNSKLCKALKYSFADY

>gi | 254952600 | gb | ACT97130.1 | VAR2CSA [Plasmodium falciparum] | 344 aa (SEQ ID NO:13)

KCDKCKSGTSTVNKKWIWKKYSGTEGGLQEEYANTIALPPRTQSLYLGNLPKLENVCKDVT
DINFDTKEKFLAGCLIAAFHEGKNLKTTYLEKKKGDNGKKNDDNNSKLCKALKYSFADYGD
LIKGTSIWDNDFTKDLELNLQQIFGKLFRKYIKKNIASDENTLYSSLDELRESWWNTNKKYIW
LAMKHGAGMNSTMCNADGSVTGSGSSCDDIPTIDLIPQYLRFLQEWVEHFCKQRQAKVKDV
ITNCNSCKECGGTCNGECKTECEKKCKGECDAYKKFIEECKGKADEGTSGSSWSKRWDQIY
KRYSKYIEDAKRNRKAGTKNCGPSSTTSTAESKCVQS

>gi | 254952598 | gb | ACT97129.1 | VAR2CSA [Plasmodium falciparum] | 334 aa (SEQ ID NO:14)

KCDKCKSEQSKKNNNIWIWKKSSGTEGGLQKEYANTIALPPRTQSLYLGNLRKLENVCEDV
KDINFDTKEKFLAGCLIAAFHEGKNLKKRYLEKKNGDNNSKLCKALKYSFADYGDLIKGTSI
WDNEYTKDLELNLQKIFGKLFRKYIKKNNTAEQHTSYSSLDELRESWWNTNKKYIWLAMK
HGTTCSSGSGDNGSISCDDIPTIDLIPQYLRFLQEWVEHFCEQRQGKVNAVIENCNSCKNTSSK
TKLGGTCNGECKTECKGECDAYKEFIEKCKGTAAEGTSGSSWVKRWYQIYMRYSKYIEDAK
RNRKAGTKNCGTSSTTSTAESKCVQS

>gi | 254952596 | gb | ACT97128.1 | VAR2CSA [Plasmodium falciparum] | 332 aa (SEQ ID NO:15)

KCDKCKSEQSKKNNNIWIWKKSSGTEGGLQKEYANTIALPPRTQSLYLGNLRKLENVCEDV
KDINFDTKEKFLAGCLIAAFHEGKNLKKRYLEKKNGDNNSKLCKALKYSFADYGDLIKGTSI
WDNEYTKDLELNLQKIFGKLFRKYIKKNNTAEQDTSYSSLDELRESWWNTNKKYIWTAMK
HGTTCSSGSGDNGSISCDDIPTIDLIPQYLRFLQEWVEHFCEQRQEKVKDVIKNCNSCKECGG
TCNGECKTECKNKCKDECDAYKKFIEECEGKAAEGTSGSSWSKRWDQIYKRYSKYIEDAKR
NRKAGTKNCGTSSTTSTAENKCVQS

>gi | 90193465 | gb | ABD92328.1 | erythrocyte membrane protein 1 [Plasmodium falciparum] | 267 aa (SEQ ID NO:16)

NYIKDDPYSAEYTTKLSFILNSSDTENASEKIQKNNDEVCNPNESGIACVELAQTSGSSSNKTC
NTHSFIKANKKKVCKDVKLGINKKDKDLKICVIEDDSLRGVDNCCCQDLLGILQENCSDKNQ
SGSSSNGSCNNKNQEACQKKLENVFASLTNGYKCEKCKSEQSKKNNKNWIWKKYSVKEEG
LQKEYANTIALPPRTQSLYLGNLPKLGNVCKGVTDINFDTKEKFLAGCLIAAFHEGKNLKTTY
LQNKKKLCKALKYSFADY

>gi | 90193477 | gb | ABD92334.1 | erythrocyte membrane protein 1 [Plasmodium falciparum] | 263 aa (SEQ ID NO:17)

DYIKGDPYFAEYATKLSFILNSSDANTSSGETANHNDEACNPNESEIASVEQASISDRSSQKAC
NTHSSIKANKKKECKHVKLGVRENDKDLKICVIEDTSLSGVDNCCCQDLLGILQENCSDNKR
GSSSNGSCDKNSEEICQKKLDEALASLHNGYKNQKCKSEQSKKNKNKWIWKKSSGNEKGLQ
KEYANTIGLPPRTQSLYLGNLPKLENVCEDVTDINFDTKEKFLAGCLIAAFHEGKNLKTTYPQ
NKNDDNGKKLCKD

>gi | 254952594 | gb | ACT97127.1 | VAR2CSA [Plasmodium falciparum] | 338 aa (SEQ ID NO:18)

KCDKCKSEQSKKNNNIWIWKKSSGNKKGLQKEYANTIGLPPRTQSLYLGNLPKLENVCKDV
TDINFDTKEKFLAGCLIAAFHEGKNLKISNEKKNDDNGKKLCKDLKYSFADYGDLIKGTSIW
DNEYTKDLELNLQNNFGKLFRKYIKKNNTAEQHTLYSSLDELRESWWNTNKKYIWLAMKH
GTTCSSGSGDNGDGSVTGSGSSCDDMSTIDLIPQYLRFLQEWVEHFCKQRQEKVNAVIENCN
SCKNTSSKTKLGGTCNGECKTECEKKCKDECEKYKEFIEECKRGDGTAGSPWVKRWDQIYM
RYSKYIEDAKRNRKAGTKSCGTSAAENKCVQS

>gi | 254952602 | gb | ACT97131.1 | VAR2CSA [Plasmodium falciparum] | 341 aa (SEQ ID NO:19)

KCDKCKSEQSKKNNNIWIWKKSSGDEKGLQKEYANTIALPPRTQSLYLGNLPKLENVCKDV
TDINFDTKEKFLAGCLIAAFHEGKNLKTSHQNKNADNGKKNDDNGKKLCKALKYSFADYG
DLIKGTSIWDNEYTKDLELNLQQIFGKLFRKYIKRNNTAEQHTLYSSLDELRESWWNTNKKY
IWLAMKHGTTCSSGSGDNGDGSVTGSGSSCDDMSTIDLIPQYLRFLQEWVEHFCKQRQEKV
KDVITNCNSCKECGGTCGSDCKTKCEAYKKFIEECNGTADGGTSGSSWSKRWDQIYKRYSK
YIEDAKRNRKAGTKNCGPSSGANSGVTTTENKCVQS

>gi | 254952660 | gb | ACT97160.1 | VAR2CSA [Plasmodium falciparum] | 352 aa (SEQ ID NO:20)

KCEKCESEQSKKNNKYWIWKKSSGNGEGLQEEYANTIALPPRTHSLCLVCLHEKEGKKTQEL
KNIRTNSELLKERIIAAFHEGKNLKTSPQNKNDNGKKLCKDLKYSFADYGDLIKGTSIWDNEY
TKDLELNLQKIFGKLFRKYIKKNNTAEQHTLYSSLDELRESWWNTNKKYIWLAMKHGAGM
NSTMCNADGSVTGSSDSGSTTCCGDNGSISCDDMPTIDLIPQYLRFLQEWVEHFCEQRQEKV
NAVITNCKSCKECGGTCNSDCEKKCKAYKEFIEKCKGGGTEGTSGSSWSKRWDQIYKRHSK
HIEDAKRNRKAGTKNCGITTGTISGESSGANSGVTTTENKCVQS

>gi | 254952652 | gb | ACT97156.1 | VAR2CSA [Plasmodium falciparum] | 344 aa (SEQ ID NO:21)

KCDKCKSGTSRSRKIWTWRKFRGNGEGLQKEYANTIGLSPRTQLLYLVCLHEKGKKTQELK
NISTNSELLKEWIIAAFHEGKNLKTTYPQKKNDDNGKKLCKALKYSFADYGDLIKGTSIWDN
DFTKDLELNLQKIFGKLFRKYIKKNIASDENTSYSSLDELRESWWNTNKKYIWTAMKHGAG
MNGTTCCGDGSVTGSSDSGSTTCCGDGSVTGSGSSCDDIPTIDLIPQYLRFLQEWVEHFCEQR
QEKVKDVITNCKSCKESEKKCKNKCDAYKEFIDGTGSGGGTGTAGSSWSKRWDQIYMRYSK
YIEDAKRNRKAGTKNCGTSSGANSGVTTTENKCVQS

>gi | 254952622 | gb | ACT97141.1 | VAR2CSA [Plasmodium falciparum] | 350 aa (SEQ ID NO:22)

KCEKCKSEQSKKNNKIWTWRKFPGNGEGLQKEYANTIGLSPRTQLLYLVCLHEKGKKTQHK
TISTNSELLKEWIIAAFHEGKNLKKRYLEKKKGDNNSKLCKDLKYSFADYGDLIKGTSIWDN
DFTKDLELNLQQIFGKLFRKYIKKNIASDENTSYSSLDELRESWWNTNKKYIWTAMKHGAG
MNSTMCNGDGSVTGSSDSGSTTCSGDNGSISCDDIPTIDLIPQYLRFLQEWVEHFCEQRQEKV
KDVIKNCNSCKECGGTCNGECKTECKNKCKDECEKYKNFIEVCTGGDGTAGSPWSKRWYQI
YMRYSKYIEDAKRNRKAGTKSCGTSSGANSGVTTTESKCVQS

>gi | 254952626 | gb | ACT97143.1 | VAR2CSA [Plasmodium falciparum] | 359 aa (SEQ ID NO:23)

KCEKCKSEQSKKNNKNWIWRKFPGNGEGLQKEYANTIGLPPRTHSLYLVCLHEKGKKTQEL
KNIRTNSELLKEWIIAAFHEGKNLKKRYHQNNNSGNKKKLCKALEYSFADYGDLIKGTSIWD
NEYTKDLELNLQQIFGKLFRKYIKKNISTEQDTLYSSLDELRESWWNTNKKYIWLAMKHGA
GMNSTTCCGDGSVTGSSDSGSTTCSGDNGSISCDDMPTIDLIPQYLRFLQEWVEHFCEQRQEK
VKDVIENCKSCKNTSGERIIGGTCNGECKTECEKKCKAACEAYKTFIEECEGKAAEGTSGSSW
SKRWYQIYMRYSKYIEDAKRNRKAGTKNCGKSSGANSGVTTTENKCVQS

>gi | 90193469 | gb | ABD92330.1 | erythrocyte membrane protein 1 [Plasmodium falciparum] | 270 aa (SEQ ID NO:24)

NYIKDDPYSKEYVTKLSFIPNSSDANNPSGETANHNDEVCNPNESEISSVEHAQTSVLLSQKA
YITHSSIKANKKKVCKYVKLGVRENDKDLKICVIEDDSLRGVENCCFKDFLRILQENCSDNKR
ESSSNGSCNNNNEEACEKNLDEALASLTNCYKNQKCKSGTSTVNNNKWIWKKSSGKEGGLQ
KEYANTIGLPPRTQSLCLVVCLDEKEGKTQELKNIRTNSELLKEWIIAAFHEGKNLKKRYHQN
KNDDNNSKLCKALKYSFADY

>gi | 254952644 | gb | ACT97152.1 | VAR2CSA [Plasmodium falciparum] | 334 aa (SEQ ID NO:25)

KCDKCKSEQSKKNNKYWIWKKYSVKEGGLQKEYANTIALPPRTQSLCLVVCLDEKEGKTQE
LKNIRTNSELLKERIIAAFHEGKNLKTYHEKKKGDDGKKLCKDLKYSFADYGDLIKGTSIWD
NDFTKDLELNLQKIFGKLFRKYIKKNNTAEQHTSYSSLDELRESWWNTNKKYIWTAMKHGA
EMNGTTCSCSGDSSNDIPTIDLIPQYLRFLQEWVEHFCEQRQAKVNAVIKNCKSCKECGGTCN
GECKTECKTKCKGECEKYKEFIEKCEGQAAEGTSGSSWSKRWYQIYMRYSKYIEDAKRNRK
AGTKNCGTSSGANSGVTTTENKCVQS

>gi | 254952642 | gb | ACT97151.1 | VAR2CSA [Plasmodium falciparum] | 351 aa (SEQ ID NO:26)

KCDKCKSEQSKKNNKNWIWKKYSGTEGGLQKEYANTIALPPRTQSLYLVCLHEKEEKTQEL
KNISTNSELLKEWIIAAFHEGKNLKISPQNKNDNGKNLCKDLKYSFADYGDLIKGTSIWDNDF
TKDLELNLQQIFGKLFRKYIKKNNTAEQDTLYSSLDELRESWWNTNKKYIWTAMKHGAGM
NGTTCCGDGSVTGSSDSGSTTCCGDGSVTGSGSSCDDIPTIDLIPQYLRFLQEWVEHFCEQRQ
AKVKDVIKNCNSCKECGGTCNGECKTECEKKCKGECEAYKKFIEKCNGGGGEGTSGSSWSK
RWDQIYMRYSKYIEDAKRNRKAGTKNCGTSSTTNAAENKCVQS

>gi | 254952658 | gb | ACT97159.1 | VAR2CSA [Plasmodium falciparum] | 353 aa (SEQ ID NO:27)

KCDKCKSGTSTVNKKWIWKKFPGKEGGLQEEYANTIALPPRTQSLCLVVCLDEKEGKTQHK
TISTNSELLKEWIIAAFHEGKNLKISNKKKNDENNSKLCKDLKYSFADYGDLIKGTSIWDNDF
TKDLELNLQKIFGKLFRKYIKKNNTAEQDTSYSSLDELRESWWNTNKKYIWLAMKHGTTCS
SGSGDNGDGSVTGSSDSGSTTCCGDGSVTGSGSSCDDIPTIDLIPQYLRFLQEWVEHFCKQRQ
AKVKDVIENCKSCKNTSSKTKLGDTCNSDCKTKCKVACEKYKEFIEKCVSAAGGTSGSSWV
KRWDQIYMRYSKYIEDAKRNRKAGTKNCGPSSTTSTAESKCVQS

>gi | 254952640 | gb | ACT97150.1 | VAR2CSA [Plasmodium falciparum] | 327 aa (SEQ ID NO:28)

KCDKCKSGTSTVNKKWIWKKYSGKEGGLQKEYANTIGLPPRTQSLCLVCLHEKEGKTQELK
NISTNSELLKEWIIAAFHEGKNLKISNKKKNDDNGKKLCKDLKYSFADYGDLIKGTSIWDNDF
TKDLELNLQKIFGKLFRKYIKKNNTAEQDTLYSSLDELRESWWNTNKKYIWTAMKHGAGM
NSTTCSCSGDSSNDIPTIDLIPQYLRFLQEWVEHFCKQRQEKVNAVITNCKSCKESGGTCNSD
CEKKCKIECEKYKNFIEKCVTAAGGTSGSSWSKRWDQIYKMYSKYIEDAKRNRKAGTKNCG
PSSTTNAAASTDENKCVQS

>dd2full 745 amino acids | 628 aa (SEQ ID NO:29)

NYIKGDPYFAEYATKLSFILNSSDTENASETPSKYYDEACNCNESEIASVGQAQTSGPSSN
KTCITHSSIKTNKKKECKDVKLGINNNDKVLRVCVIEDTSLSGVDNCCCQDLLGILQEN
CSDNKRGSSSNGSCDKNSEEICQKKLEKVFASLTNGYKCDKCKSGTSRSKKKWIWKKSS
GNEEGLQKEYANTIGLPPRTQSLCLVCLHEKEGKTQHKTISTNSELLKEWIIAAFHEGK
NLKTSHEKKNDDNGKKLCKALEYSFADYGDLIKGTSIWDNEYTKDLELNLQKIFGKLF
RKYIKKNNTAEQHTSYSSLDELRESWWNTNKKYIWTAMKHGAGMNGTTCSCSGDSSN
DMPTIDLIPQYLRFLQEWVEHFCKQRQEKVNAVIENCNSCKESGGTCNSDCKTECKNK
CEAYKEFIEDCKGGGTGTAGSPWSKRWDQIYKRYSKHIEDAKRNRKAGTKNCGTSSTT
NAAASTDENKCVQSDVDSFFKHLIDIGLTTPSSYLSNVLDDNICGADKAPWTTYTTYTTT

KNCDIQKKTPKSQSCDTLVVVNVPSPLGNTPHEYKYACECKIPTTEETCDDRKEYMNQWS CGSAQTVRGRSGKDDYELYTYNGVKETKPLGTLKNSKLD

>gi | 254952636 | gb | ACT97148.1 | VAR2CSA [Plasmodium falciparum] | 350 aa (SEQ ID NO:30)

KCEKCKSEQSKKNNKNWIWRKFRGTEGGLQEEYANTIGLPPRTQSLCLVVCLDEKGKKTQE LKNIRTNSELLKEWIIAAFHEGKNLKPSHQNKNSGNKENLCKALKYSFADYGDLIKGTSIWD NDFTKDLELNLQKIFGKLFRKYIKKNNTAEQHTSYSSLDELRESWWNTNKKYIWTAMKHGA EMNGTTCNADGSVTGSSDSGSTTCSGDNGSISCDDIPTIDLIPQYLRFLQEWVEHFCKQRQEK VNAVINSCNSCKNTSSKTKLGDTCNSDCKTKCKIECEKYKTFIEKCVTAAGGTSGSPWSKRW DQIYKRYSKYIEDAKRNRKAGTKNCGPSSTTSTAESKCVQS

>gi | 254952638 | gb | ACT97149.1 | VAR2CSA [Plasmodium falciparum] | 330 aa (SEQ ID NO:31)

KCDKCKSEQSKKNNKNWIWRKYSGNGEGLQKEYANTIGLPPRTHSLYLVCLHEKEGKTQEL KNIRTNSELLKEWIIAAFHEGKNLKTTYLENKNDENKKKLCKALKYSFADYGDLIKGTSIWD NDFTKDLELNLQKIFGKLFRKYIKKNIASDENTLYSSLDELRESWWNTNKKYIWTAMKHGAE MNGTTCSSGSGDNGSISCDDIPTIDLIPQYLRFLQEWVGHFCKQRQEKVNAVITNCNSCKESG GTCNSDCEKKCKIECEKYKKFIEECRTAAGGTSGSPWSKRWDQIYKMYSKYIEDAKRNRKA GTKNCGPSSTTSTAESKCVQS

>gi | 254952628 | gb | ACT97144.1 | VAR2CSA [Plasmodium falciparum] | 334 aa (SEQ ID NO:32)

KCDKCKSEQSKKNNKNWIWRKYSGNGEGLQKEYANTIGLPPRTHSLYLVCLHEKEGKTQHK TISTNSELLKEWIIAAFHEGKNLKKRYPQNNNSGNKKKLCKDLKYSFADYGDLIKGTSIWDN EYTKDLELNLQKAFGKLFRKYIKKNIASDENTLYSSLDELRESWWNTNKKYIWLAMKHGAE MNGTMCNADGSVTGSGSSCDDMSTIDLIPQYLRFLQEWVEHFCEQRQAKVKDVINSCKSCK ESGDTCNSDCEKKCKNKCDAYKTFIEEFCTADGGTAGSPWSKRWDQIYKRYSKYIEDAKRN RKAGTKNCGTSSGANSGVTTTENKCVQS

>gi | 254952630 | gb | ACT97145.1 | VAR2CSA [Plasmodium falciparum] | 350 aa (SEQ ID NO:33)

KCDKCKSGTSTVNKNWIWKKYSGKEEGLQKEYANTIALPPRTHSLYLVCLHEKGKKTQELK NIRTNSELLKEWIIAAFHEGKNLKTSPQNNNSGNKKKLCKALKYSFADYGDLIKGTSIWDND FTKDLELNLQKIFGKLFRKYIKKNNTAEQHTSYSSLDELRESWWNTNKKYIWLAMKHGAEM NGTTCCGDGSVTGSSDSGSTTCSGDNGSISCDDMPTTDFIPQYLRFLQEWVEHFCKQRQEKV

KHVMESCKSCKECGDTCNGECKTECEKKCKNKCEAYKTFIEKCVSADGGTSGSSWSKRWD
QIYMRYSKYIEDAKRNRKAGTKNCGTSSTTNAAASTAENKCVQS

>P13 745 amino acids | 647 aa (SEQ ID NO:34)

DYIKDDPYSAEYATKLSFILNPSDANTSSGETANHNDEVCNCNESEIASVELAPISDSSSNK
TCITHSFIGANKKKECKDVKLGVREKDKDLKICVIEDDSLRGVENCCCQDLLGILQENC
SDNKSGSSSNGSCDKNSEDECQKKLENVFASLKNGYKCDKCKSGTSTVNKKWIWRKYS
GNGEGLQKEYANTIGLPPRTHSLYLVCLHEKEGKTQHKTISTNSELLKEWIIAAFHEGK
NLKTSHQNNNSGNKKKLCKALKYSFADYGDLIKGTSIWDNDFTKDLELNLQKIFGKLF
RKYIKKNIASDENTSYSSLDELRESWWNTNKKYIWLAMKHGAEMNSTMCNGDGSVTG
SSDSGSTTCSGDNGSISCDDIPTIDLIPQYLRFLQEWVEHFCKQRQEKVKDVITNCKSCKE
SGDTCNSDCEKKCKNKCEAYKKFIEERRTAAQGTAESSWVKRWDQIYMRYSKYIEDAK
RNRKAGTKSCGPSSTTNAAASTAENKCVQSDIDSFFKHLIDIGLTTPSSYLSIVLDDNICG
ADNAPWTTYTTYTTTKNCDIKKKTPKPQSCDTLVVVNVPSPLGNTPHEYKYACQCRTPN
KQESCDDRKEYMNQWSSGSAQTVRGRSTNNDYELYTYNGVKETKPLGTLKNSKLD

>gi | 254952608 | gb | ACT97134.1 | VAR2CSA [Plasmodium falciparum] | 341 aa (SEQ ID NO:35)

KCDKCKSGTSTVNKKWIWRKSSGNKEGLQKEYANTIGLPPRTQSLYLGNLPKLENVCEDVK
DINFDTKEKFLAGCLIVSFHEGKNLKTSHEKKNDDNGKKLCKALEYSFADYGDLIKGTSIWD
NEYTKDLELNLQKIFGKLFRKYIKKNNTAEQDTSYSSLDELRESWWNTNKKYIWTAMKHGA
GMNITTCCGDGSSGENQTNSCDDIPTIDLIPQYLRFLQEWVEHFCKQRQEKVNAVVTNCKSC
KESGGTCNGECKTKCKNKCEVYKTFIDNVGDGTAGSPWVKRWDQIYKRYSKHIEDAKRNR
KAGTKNCGITTGTISGESSGATSGVTTTENKCVQS

>7g8 745 amino acids | 632 aa (SEQ ID NO:36)

NYIKDDPYSKEYVTKLSFIPNSSDANTSSEKIQKNNDEVCNPNESGISSVEQAQTSGPSSNK
TCITHSSIKANKKKECKDVKLGVRENDKDLKICVIEDTSLSGVDNCCCQDLLGILQENCS
DNKRGSSSNDSCDNKNQDECQKKLDEALESLHNGYKNQKCKSGTSTVNKKWIWKKSS
GNKEGLQKEYANTIGLPPRTQSLYLGNLPKLENVSKGVTDIIYDTKEKFLAGCLIVSFHE
GKNLKTSHEKKNDDNGKKLCKALEYSFADYGDLIKGTSIWDNEYTKDLELNLQKAFGK
LFRKYIKKNISAEQDTSYSSLDELRESWWNTNKKYIWIAMKHGAGMNGTTCCGDGSSG
ENQTNSCDDIPTIDLIPQYLRFLQEWVEHFCEQRQAKVKDVITNCKSCKNTSGERKIGG
TCNGECKTKCKNKCEAYKTFIEHCKGGDGTAGSSWVKRWDQIYKRYSKHIEDAKRNR
KAGTKSCGTSTAENKCVQSDIDSFFKHLIDIGLTTPSSYLSIVLDENNCGEDKAPWTTYT

TTKNCDIQKDKSKSQSSDTLVVVNVPSPLGNTPHGYKYACQCKIPTTEETCDDRKEYMNQ
WSCGSARTMKRGYKNDNYELCKYNGVDVKPTTVRSSSTKLD

>Indo 745 amino acids | 639 aa (SEQ ID NO:37)

DYIKGDPYSAEYVTKLSFIPNSSDANNPSEKIQKNNDEVCNCNESEISSVGQASISDPSSNK
TCNTHSSIKANKKKVCKDVKLGVRENDKVLKICVIEHTSLRGVDNCCFKDLLGILQEPR
IDKNQSGSSSNGSCDKNSEEACEKNLEKVLASLTNGYKCDKCKSGTSRSKKKWIWKKY
SGKEGGLQEEYANTIGLPPRTQSLCLVVCLDEKEGKTQELKNISTNSELLKEWIIAAFPE
GKNLKPSPEKKKGDNGKKLCKDLKYSFADYGDLIKGTSIWDNEYTKDLELNLQKIFGK
LFRKYIKKNIASDENTLYSSLDELRESWWNTNKKYIWLAMKHGAGMNSTMCNADGSV
TGSGSSCDDMPTIDLIPQYLRFLQEWVEHFCKQRQEKVKPVIENCNSCKNTSSERKIGG
TCNSDCKTECKNKCEVYKKFIEDCKGGDGTAGSSWSKRWDQIYKRYSKYIEDAKRNRK
AGTKNCGPSSTTNAAENKCVQSDIDSFFKHLIDIGLTTPSSYLSTVLDDNICGEDNAPWTT
YTTYTTTKNCDKDKKKSKSQSCDTLVVVNVPSPLGNTPHEYKYACECRTPNKQESCDDR
KEYMNQWISDNTKNPKGSGSGKDYYELYTYNGVDVKPTTVRSSSTKLD

>MC 745 amino acids | 655 aa (SEQ ID NO:38)

DYIKGDPYFAEYATKLSFILNSSDANTSSGETANHNDEACNCNESEISSVEHASISDPSSNK
TCNTHSSIKANKKKVCKHVKLGVRENDKDLRVCVIEHTSLSGVENCCFKDFLRILQENC
SDNKSGSSSNGSCDKNNEEACEKNLEKVFASLTNCYKCEKCKSEQSKKNNKKWTWRKS
SGNKGGLQEEYANTIGLPPRTQSLCLVVCLDEKEGKKTQELKNIRTNSELLKEWIIAAF
HEGKNLKPSHEKKNDDNGKKNDDNNSKLCKDLKYSFADYGDLIKGTSIWDNEYTKDLE
LNLQKIFGKLFRKYIKKNIASDENTLYSSLDELRESWWNTNKKYIWLAMKHGAEMNGT
TCNADGSVTGSGSSCDDIPTIDLIPQYLRFLQEWVEHFCKQRQAKVKDVIENCKSCKES
GNKCKTECKNKCEAYKKFIENCKGGDGTAGSSWVKRWDQIYMRYSKYIEDAKRNRKA
GTKNCGPSSITNVSASTDENKCVQSDIDSFFKHLIDIGLTTPSSYLSIVLDDNICGDDKAPW
TTYTTYTTYTTYTTYTTYTTYTTTKNCDKERDKSKSQSCNTAVVVNVPSPLGNTPHEYK
YACECRTPSNKELCDDRKEYMNQWSSGSAQTVRDRSGKDYYELYTYNGVKETKLPKKLNS
SKLD

>gi | 254952650 | gb | ACT97155.1 | VAR2CSA [Plasmodium falciparum] | 347 aa (SEQ ID NO:39)

KCDKCKSEQSKKNNKYWIWKKSSVKEEGLQKEYANTIALPPRTHSLCLVVCLDEKGKKTQE
LKNISTNSELLKERIIAAFHEGKNLKTTYLEKKNADNNSKLCKALKYSFADYGDLIKGTSIWD
NEYTKDLELNLQQIFGKLFRKYIKKNNTAEQHTLYSSLDELRESWWNTNKKYIWLAMKHGA
GMNGTTCCGDGSVTGSSDSGSTTCSGDNGSISCDDMPTTDFIPQYLRFLQEWVEHFCKQRQE

KVKDVIENCNSCKNNLGKTEINEKCKTECKNKCEAYKNFIEKFCTADGGTSGSPWSKRWDQI
YKRYSKYIEDAKRNRKAGTKNCGTSSTTSTAENKCVQS

>gi | 254952648 | gb | ACT97154.1 | VAR2CSA [Plasmodium falciparum] | 335 aa (SEQ ID NO:40)

KCEKCKSGTSTVNKYWIWRKSSGNKEGLQKEYANTIALPPRTHSLCLVVCLDEKEGKTQEL
KNISTNSELLKERIIAAFHEGENLKTSHEKKKGDDGKKNADNNSKLCKALKYSFADYGDLIK
GTSIWDNEYTKDLELNLQKIFGKLFRKYIKKNIASDENTSYSSLDELRESWWNTNKKYIWLA
MKHGAGMNGTTCSCSGDSSDDMPTTDFIPQYLRFLQEWVEHFCKQRQENVNAVIENCNSCK
ECGGTCNSDCEKKCKTECKNKCEAYKNFIEKFCTADGGTSGYSWSKRWDQIYKRYSKYIED
AKRNRKAGTKSCGTSSTTSTAESKCVQS

>ghana2 745 amino acids | 667 aa (SEQ ID NO:41)

**SYVKNNPYSKEYVTKLSFILNPSDANNPSETPSKYYDEVCNCNESGIACVGQAQTSGPSS
NKTCITHSFIGANKKKVCKDVKLGVREKDKDLKICVIEDTYLSGVDNCCFKDFLGMLQ
ENCSDNKSGSSSNGSCNNKNQDECEKNLDEALASLTNGYKCEKCKSGTSTVNKYWIWR
KSSGNKEGLQKEYANTIALPPRTHSLCLVVCLDEKEGKTQHKTISTNSELLKEWIIAAFH
EGKNLKTSHEKKKGDDGKKNADNNSKLCKALKYSFADYGDLIKGTSIWDNDFTKDLEL
NLQKIFGKLFRKYIKKNIASDENTSYSSLDELRESWWNTNKKYIWLAMKHGAGMNSTT
CCGDGSVTGSSDSGSTTCCGDGSVTGSGSSCDDMPTTDFIPQYLRFLQEWVEHFCKQRQ
ENVNAVIENCNSCKECGGTCNSDCEKKCKTECKGECDAYKEFIEKCNGGAAEGTSGSS
WSKRWDQIYKRYSKYIEDAKRNRKAGTKNCGTSSTTSTAESKCVQSDIDSFFKHLIDIGL
TTPSSYLSIVLDENICGADNAPWTTYTTYTTYTTYTTTEKCNKETDKSKLQQCNTSVVV
NVPSPLGNTPHGYKY**VCECRTPNKQETCDDRKEYMNQWISDNTKNPKGSRSTNNDYELYT
YNGVQIKPTTVRSNSTKLD

>gi | 254952634 | gb | ACT97147.1 | VAR2CSA [Plasmodium falciparum] | 348 aa (SEQ ID NO:42)

KCDKCKSEQSKKNNKNWIWKKSSGNEKGLQKEYANTIGLPPRTQSLCLVVCLDEKEGKTQE
LKNIRTNSELLKEWIIAAFHEGKNLKTSHEKKKGDNNSKLCKDLKYSFADYGDLIKGTSIWD
NEYTKDLELNLQNNFGKLFRKYIKKNIASDENTSYSSLDELRESWWNTNKKYIWLAMKHGA
GMNSTTCSSGSGSTTCSSGSGSTTCSSGSGDSCDDMPTIDLIPQYLRFLQEWVEHFCKQRQEK
VNAVIKNCNSCKESGGTCNGECKTECKNKCEAYKTFIEEFCTADGGTSGSPWSKRWDQIYK
MYSKHIEDAKRNRKAGTKNCGPSSTTNVSVSTDENKCVQS

>ghana1 745 amino acids | 652 aa (SEQ ID NO:43)

DYIKDDPYFAEYVTKLSFILNSSDANNPSGETANHNDEVCNPNESGIASVEQAQTSDPSSN
KTCNTHSSIKANKKKVCKHVKLGVRENDKDLKICVIEHTSLSGVENCCCQDFLRILQEN
CSDNKSGSSSNGSCNNKNQEACEKNLEKVLASLTNCYKCDKCKSEQSKKNNKNWIWK
KSSGNEKGLQKEYANTIGLPPRTQSLCLVVCLDEKEGKTQELKNIRTNSELLKEWIIAAF
HEGKNLKKRYPQNKNDDNNSKLCKDLKYSFADYGDLIKGTSIWDNEYTKDLELNLQNN
FGKLFRKYIKKNISTEQDTLYSSLDELRESWWNTNKKYIWLAMKHGAGMNSTTCSSGS
GSTTCSSGSGSTTCSSGSGDSCDDMPTTDFIPQYLRFLQEWVEHFCKQRQEKVNAVIKN
CNSCKESGGTCNGECKTECKNKCEAYKTFIEEFCTADGGTSGSPWSKRWDQIYKMYSK
HIEDAKRNRKAGTKNCGPSSTTNVSVSTDENKCVQSDIDSFFKHLIDIGLTTPSSYLSIVL
DDNICGEDKAPWTTYTTYTTTKKCNKETDKSKSQSCNTAVVVNVPSPLGNTPHGYKYA
CECKIPTTEETCDDRKEYMNQWIIDTSKKQKGSGSGKDDYELYTYNGVDVKPTTVRSNSTKL
D

>V1S1 745 amino acids | 628 aa (SEQ ID NO:44)

DYIKDDPYSAQYTTKLSFILNPSDANTSSEKIQKNNDEACNCNESGISSVGQAQTSGPSSN
KTCITHSSIKANKKKVCKDVKLGINNNDKVLRVCVIEDTSLSGVDNCCCQDLLGILQEN
CSDNKRGSSSNGSCNNNNEEACEKNLDEAPASLHNGYKNQKCKSGTSRSKKKWIWKKS
SGNEKGLQEEYANTIGLPPRTQSLCLVCLHEKEGKTQHKTISTNSELLKEWIIAAFHEG
KNLKTSHEKKNDDNGKKLCKALEYSFADYGDLIKGTSIWDNEYTKDLELNLQKAFGKL
FRKYIKKNNTAEQDTSYSSLDELRESWWNTNKKYIWIAMKHGAGMNGTTCSCSGDSSN
DMPTIDLIPQYLRFLQEWVEHFCEQRQAKVKDVITNCKSCKESGNKCKTECKTKCKDE
CEKYKTFIEDCNGGGTGTAGSSWVKRWDQIYKRYSKHIEDAKRNRKAGTKNCGPSSIT
NAAASTDENKCVQSDIDSFFKHLIDIGLTTPSSYLSNVLDENSCGDDKAPWTTYTTYTTT
KNCDIQKDKSKSQPINTSVVVNVPSPLGNTPYRYKYACECKIPTTEESCDDRKEYMNQWS
CGSARTMKRGYKNDNYELCKYNGVDVKPTTVRSNSSKLD

>raj116_var25 745 amino acids | 653 aa (SEQ ID NO:45)

DYIKGDPYFAEYATKLSFILNPSDTENASETPSKYYDEACNPNESEIASVEQAQTSGPSSN
KTCITHSSIKTNKKKECKDVKLGVRENDKDLKICVIEDTSLSGVDNCCFKDLLGILQENC
SDNKRGSSSNDSCNNNNEEACEKNLDEALASLTNGYKCDKCKSGTSTVNKKWTWRKSS
GNEEGLQKEYANTIGLPPRTQSLCLVCLHEKEGKTKHKTISTNSELLKEWIIAAFHEGK
NLKTSHEKKNDDNGKKLCKALEYSFADYGDLIKGTSIWDNEYTKDLELNLQKAFGKLF
RKYIKKNNTAEQDTSYSSLDELRESWWNTNKKYIWTAMKHGAEMNGTTCSSGSGDNG
DSSITGSSDSGSTTCSGDNGSISCDDIPTTDFIPQYLRFLQEWVEHFCEQRQAKVKDVINS
CNSCNESGGTCNGECKTKCKDECEKYKKFIEDCNGGDGTAGSSWVKRWDQIYKRYSK
HIEDAKRNRKAGTKNCGPSSITNAAASTDENKCVQSDVDSFFKHLIDIGLTTPSSYLSIVL

DENSCGDDKAPWTTYTTYTTTEKCNKERDKSKSQSSDTLVVVNVPSPLGNTPHEYKYA
CECKIPTNEETCDDRKDYMNQWISDTSKKQKGSGSGKDYYELYTYNGVQIKQAAGRSSSTK
LD

>gi | 31323048 | gb | AAP37940.1 | var2csa [Plasmodium falciparum] | 490 aa (SEQ ID NO:46)

KCDKCKSEQSKKNNNKWIWKKYSGNGEGLQKEYANTIGLPPRTQSLCLVCLHEKEGKTQHK
TISTNSELLKEWIIAAFHEGKNLKKRYPQNKNDDNNSKLCKALEYSFADYGDLIKGTSIWDNE
YTKDLELNLQKAFGKLFRKYIKKNNTAEQDTSYSSLDELRESWWNTNKKYIWTAMKHGAE
MNGTTCSSGSGDNGDSSCDDIPTIDLIPQYLRFLQEWVEHFCKQRQAKVKDVINSCNSCKNTS
GERKIGGTCNSDCEKKCKVACDAYKTFIEECRTAVGGTAGSSWVKRWDQIYKRYSKHIEDA
KRNRKAGTKNCGPSSTTNAAENKCVQSDIDSFFKHLIDIGLTTPSSYLSNVLDENSCGADKAP
WTTYTTYTTYTTYTTYTTTEKCNKERDKSKSQQSNTSVVVNVPSPLGNTPHEYKYACECKIP
TTEETCDDRKEYMNQWIIDNTKNPKGSGSTDNDYELYTYNGVQIKQAAGRSSSTKLD

>gi | 254952620 | gb | ACT97140.1 | VAR2CSA [Plasmodium falciparum] | 335 aa (SEQ ID NO:47)

KCEKCKSGTSTVNNKWIWRKSSGKEGGLQKEYANTIGLPPRTQSLYLGNLPKLENVCKGVT
DIIYDTKEKFLSGCLIAAFHEGKNLKTTYLEKKNDDNGKKLCKALEYSFADYGDLIKGTSIWD
NEYTKDLELNLQKIFGKLFRKYIKKNNTAEQDTSYSSLDELRESWWNTNKKYIWIAMKHGA
GMNGTTCSSGSGDSSNDIPTTDFIPQYLRFLQEWVENFCEQRQAKVKPVIENCNSCKESGGTC
NGECKTKCKVACDAYKKFIDGTGSGGGSRPTGIAGSSWSKRWDQIYKRYSKHIEDAKRNRK
AGTKNCGPSSITNVSVSTDENKCVQS

>T2C6 745 amino acids | 637 aa (SEQ ID NO:48)

**NYIKDDPYSKEYVTKLSFIPNSSDANTSSEKIQKNNDEVCNPNESGISSVEQAQTSDPSSNK
TCITHSSIKANKKKECKDVKLGVRENDKDLKICVIEHTSLSGVDNCCFKDFLRMLQEPRI
DKNQRGSSSNGSCDKNSEEACEKNLDEALASLTNGYKCDKCKSEQSKKNNNKWIWKK
FPGKEGGLQEEYANTIGLPPRTQYLCLVVCLDEKEGKTQELKNIRTNSELLKEWIIAAF
HEGKNLKTTYPQKKNDDNGKKLCKDLKYSFADYGDLIKGTSIWDNEYTKNVELNLQN
NFGKLFRKYIKKNNTAEQDTSYSSLDELRESWWNTNKKYIWLAMKHGAEMNSTTCCG
DGSVTGSGSSCDDIPTIDLIPQYLRFLQEWVEHFCKQRQAKVKDVITNCNSCKESGNKC
KTECKNKCKDECEKYKKFIEACGTAVGGTGTAGSPWSKRWDQIYKRYSKHIEDAKRN
RKAGTKNCGPSSTTNAAENKCVQSDIDSFFKHLIDIGLTTPSSYLSIVLDDNICGADKAPW
TTYTTYTTENCDIQKKTPKSQSCDTLVVVNVPSPLGNTPHGYKY**ACQCRTPNKQESCDD
RKEYMNQWIIDNTKNPKGSGSGKDYYELCKYNGVKETKPLGTLKNSKLD

>gi | 254952632 | gb | ACT97146.1 | VAR2CSA [Plasmodium falciparum] | 330 aa (SEQ ID NO:49)

KCDKCKSEQSKKNNNKWIWRKFPGKEGGLQKEYANTIGLPPRTQSLCLVCLHEKEGKTQHK
TISTNSELLKEWIIAAFHEGKNLKTTYLEKKNAENKKKLCKALKYSFADYGDLIKGTSIWDNE
YTKDLELNLQKIFGKLFRKYIKKNNTAEQDTSYSSLDELRESWWNTNKKYIWTAMKHGAG
MNGTMCNADGSVTGSGSSCDDMPTTDFIPQYLRFLQEWVEHFCKQRQAKVKDVIENCKSCK
ESGNKCKTECKNKCDAYKTFIEECGTAVGGTAGSSWVKRWDQIYKRYSKHIEDAKRNRKA
GTKNCGTSSTTNAAASTAENKCVQS

>gi | 90193487 | gb | ABD92339.1 | erythrocyte membrane protein 1 [Plasmodium falciparum] | 269 aa (SEQ ID NO:50)

NYIKDDPYSKEYVTKLSFILNSSDAENASETPSKYYDEACNCNESGISSVEQASISDRSSQKAC
NTHSFIGANKKKVCKHVKLGVRENDKDLKICVIEDDSLRGVENCCFKDFLRMLQEPRIDKNQ
RGSSSNDSCNNNNEEACEKNLDEALASLHNGYKNQKCKSEQSKKNNNKWIWKKSSGKEGG
LQKEYANTIGLPPRTQSLCLVCLHEKEGKTQHKTISTNSELLKEWIIDAFHEGKNLKTTYLEK
KKGDNGKKLCKALKYSFADY

>gi | 254952646 | gb | ACT97153.1 | VAR2CSA [Plasmodium falciparum] | 347 aa (SEQ ID NO:51)

KCDKCKSEQSKKNNKNWIWKKSSGKEGGLQKEYANTIALPPRTQSLCLVVCLHEKEGKTQH
KTISTNSELLKEWIIDAFHEGKNLKTTYLEKQNADNGKKNADNNSKLCKDLKYSFADYGDLI
KGTSIWDNEYTKDLELNLQQIFGKLFRKYIKKNIASDENTLYSSLDELRESWWNTNKKYIWT
AMKHGAEMNGTTCSSGSGDSSSGENQTNSCDDIPTIDLIPQYLRFLQEWVEHFCEQRQAKVK
DVITNCKSCKESGGTCNSDCKTKCKGECEKYKKFIEKCKGGGTEGTSGSSWVKRWYQIYMR
YSKYIEDAKRNRKAGTKSCGTSSGANSGVTTTESKCVQS

>gi | 90193485 | gb | ABD92338.1 | erythrocyte membrane protein 1 [Plasmodium falciparum] | 269 aa (SEQ ID NO:52)

DYIKDDPYSKEYTTKLSFILNSSDANTSSEKIQKNNDEVCNPNESEISSVEQAQTSRPSSNKTCI
THSSIKANKKKVCKDVKLGVRENDKVLRVCVIEHTSLSGVENCCCQDLLGILQENCSDNKRG
SSSNGSCDKNSEEACEKNLDEALASLTNCYKNQKCKSEQSKKNNNKWIWKKSSGNEKGLQK
EYANTIGLPPRTQSLCLVCLHEKEGKTQELKNISTNSELLKEWIIAAFHEGKNLKTTYPQNKN
DDNGKKLFKDLKYSFADY

>MTS1 745 amino acids | 646 aa (SEQ ID NO:53)

**DYIKDDPYSKEYTTKLSFILNSSDANTSSEKIQKNNDEVCNPNESEISSVEQAQTSRPSSNK
TCITHSSIKANKKKVCKDVKLGVRENDKVLRVCVIEHTSLSGVENCCCQDLLGILQENC**

SDNKRGSSSNGSCDKNSEEACEKNLDEALASLTNCYKNQKCKSEQSKKNNNKWIWKKS
SGKEGGLQKEYANTIGLPPRTQSLYLGNLPKLENVCKGVTDINFDTKEKFLAGCLIAAF
HEGKNLKTTYLEKKNDDNGKKLCKALEYSFADYGDLIKGTSIWDNEYTKDLELNLQKA
FGKLFRKYIKKNNTAEQDTSYSSLDELRESWWNTNKKYIWTAMKHGAGMNGTTCSSG
SGDSSNDIPTTDFIPQYLRFLQEWVENFCEQRQAKVKDVIENCNSCKNTSGERKIGDTCN
SDCEKKCKDECEKYKKFIEDCKGGDGTAGSSWVKRWDQIYKRYSKHIEDAKRNRKAG
TKNCGITTGTISGESSGATSGVTTTENKCVQSDIDSFFKHLIDIGLTTPSSYLSNVLDDNIC
GEDNAPWTTYTTYTTEKCNKETDKSKSQQSNTAVVVNVPSPLGNTPHGYKYACECKIPT
TEETCDDRKEYMNQWSCGSAQTVRDRSGKDDYELCKYNGVQIKQAAGTLKNSKLD

>Q8I639 (Q8I639_PLAF7) Plasmodium falciparum (isolate 3D7), 632 aa extracellular part (SEQ ID NO:54)

NYIKGDPYFAEYATKLSFILNSSDANNPSEKIQKNNDEVCNCNESGIASVEQEQISDPSSN
KTCITHSSIKANKKKVCKHVKLGVRENDKDLRVCVIEHTSLSGVENCCCQDFLRILQEN
CSDNKSGSSSNGSCNNKNQEACEKNLEKVLASLTNCYKCDKCKSEQSKKNNKNWIWK
KSSGKEGGLQKEYANTIGLPPRTQSLCLVVCLDEKGKKTQELKNIRTNSELLKEWIIAA
FHEGKNLKPSHEKKNDDNGKKLCKALEYSFADYGDLIKGTSIWDNEYTKDLELNLQKI
FGKLFRKYIKKNNTAEQDTSYSSLDELRESWWNTNKKYIWLAMKHGAGMNSTTCCGD
GSVTGSGSSCDDIPTIDLIPQYLRFLQEWVEHFCKQRQEKVKPVIENCKSCKESGGTCNG
ECKTECKNKCEVYKKFIEDCKGGDGTAGSSWVKRWDQIYKRYSKYIEDAKRNRKAGT
KNCGPSSTTNAAENKCVQSDIDSFFKHLIDIGLTTPSSYLSIVLDDNICGADKAPWTTYTT
YTTTEKCNKETDKSKLQQCNTAVVVNVPSPLGNTPHGYKYACQCKIPTNEETCDDRKEY
MNQWSCGSARTMKRGYKNDNYELCKYNGVDVKPTTVRSNSSKLD

>Q8I639 (Q8I639_PLAF7) Plasmodium falciparum (isolate 3D7), complete 2730 aa extracellular part (SEQ ID NO:55)

MDKSSIANKIEAYLGAKSDDSKIDQSLKADPSEVQYYGSGGDGYYLRKNICKITVNHSDSGT
NDPCDRIPPPYGDNDQWKCAIILSKVSEKPENVFVPPRRQRMCINNLEKLNVDKIRDKHAFLA
DVLLTARNEGERIVQNHPDTNSSNVCNALERSFADIADIIRGTDLWKGTNSNLEQNLKQMFA
KIRENDKVLQDKYPKDQNYRKLREDWWNANRQKVWEVITCGARSNDLLIKRGWRTSGKSN
GDNKLELCRKCGHYEEKVPTKLDYVPQFLRWLTEWIEDFYREKQNLIDDMERHREECTSED
HKSKEGTSYCSTCKDKCKKYCECVKKWKSEWENQKNKYTELYQQNKNETSQKNTSRYDD
YVKDFFKKLEANYSSLENYIKGDPYFAEYATKLSFILNSSDANNPSEKIQKNNDEVCNCNESG
IASVEQEQISDPSSNKTCITHSSIKANKKKVCKHVKLGVRENDKDLRVCVIEHTSLSGVENCC
CQDFLRILQENCSDNKSGSSSNGSCNNKNQEACEKNLEKVLASLTNCYKCDKCKSEQSKKN
NKNWIWKKSSGKEGGLQKEYANTIGLPPRTQSLCLVVCLDEKGKKTQELKNIRTNSELLKE

WIIAAFHEGKNLKPSHEKKNDDNGKKLCKALEYSFADYGDLIKGTSIWDNEYTKDLELNLQ
KIFGKLFRKYIKKNNTAEQDTSYSSLDELRESWWNTNKKYIWLAMKHGAGMNSTTCCGDGS
VTGSGSSCDDIPTIDLIPQYLRFLQEWVEHFCKQRQEKVKPVIENCKSCKESGGTCNGECKTE
CKNKCEVYKKFIEDCKGGDGTAGSSWVKRWDQIYKRYSKYIEDAKRNRKAGTKNCGPSSTT
NAAENKCVQSDIDSFFKHLIDIGLTTPSSYLSIVLDDNICGADKAPWTTYTTYTTTEKCNKETD
KSKLQQCNTAVVVNVPSPLGNTPHGYKYACQCKIPTNEETCDDRKEYMNQWSCGSARTMK
RGYKNDNYELCKYNGVDVKPTTVRSNSSKLDDKDVTFFNLFEQWNKEIQYQIEQYMTNTKI
SCNNEKNVLSRVSDEAAQPKFSDNERDRNSITHEDKNCKEKCKCYSLWIEKINDQWDKQKD
NYNKFQRKQIYDANKGSQNKKVVSLSNFLFFSCWEEYIQKYFNGDWSKIKNIGSDTFEFLIKK
CGNDSGDGETIFSEKLNNAEKKCKENESTNNKMKSSETSCDCSEPIYIRGCQPKIYDGKIFPGK
GGEKQWICKDTIIHGDTNGACIPPRTQNLCVGELWDKRYGGRSNIKNDTKESLKQKIKNAIQ
KETELLYEYHDKGTAIISRNPMKGQKEKEEKNNDSNGLPKGFCHAVQRSFIDYKNMILGTSV
NIYEYIGKLQEDIKKIIEKGTTKQNGKTVGSGAENVNAWWKGIEGEMWDAVRCAITKINKKQ
KKNGTFSIDECGIFPPTGNDEDQSVSWFKEWSEQFCIERLQYEKNIRDACTNNGQGDKIQGDC
KRKCEEYKKYISEKKQEWDKQKTKYENKYVGKSASDLLKENYPECISANFDFIFNDNIEYKT
YYPYGDYSSICSCEQVKYYEYNNAEKKNNKSLCHEKGNDRTWSKKYIKKLENGRTLEGVYV
PPRRQQLCLYELFPIIIKNKNDITNAKKELLETLQIVAEREAYYLWKQYHAHNDTTYLAHKKA
CCAIRGSFYDLEDIIKGNDLVHDEYTKYIDSKLNEIFDSSNKNDIETKRARTDWWENEAIAVP
NITGANKSDPKTIRQLVWDAMQSGVRKAIDEEKEKKKPNENFPPCMGVQHIGIAKPQFIRWL
EEWTNEFCEKYTKYFEDMKSNCNLRKGADDCDDNSNIECKKACANYTNWLNPKRIEWNGM
SNYYNKIYRKSNKESEDGKDYSMIMEPTVIDYLNKRCNGEINGNYICCSCKNIGENSTSGTVN
KKLQKKETQCEDNKGPLDLMNKVLNKMDPKYSEHKMKCTEVYLEHVEEQLKEIDNAIKDY
KLYPLDRCFDDKSKMKVCDLIGDAIGCKHKTKLDELDEWNDVDMRDPYNKYKGVLIPPRRR
QLCFSRIVRGPANLRNLKEFKEEILKGAQSEGKFLGNYYNEDKDKEKALEAMKNSFYDYEYII
KGSDMLTNIQFKDIKRKLDRLLEKETNNTEKVDDWWETNKKSIWNAMLCGYKKSGNKIIDP
SWCTIPTTETPPQFLRWIKEWGTNVCIQKEEHKEYVKSKCSNVTNLGAQESESKNCTSEIKKY
QEWSRKRSIQWEAISEGYKKYKGMDEFKNTFKNIKEPDANEPNANEYLKKHCSKCPCGFND
MQEITKYTNIGNEAFKQIKEQVDIPAELEDVIYRLKHHEYDKGNDYICNKYKNINVNMKKNN
DDTWTDLVKNSSDINKGVLLPPRRKNLFLKIDESDICKYKRDPKLFKDFIYSSAISEVERLKKV
YGEAKTKVVHAMKYSFADIGSIIKGDDMMENNSSDKIGKILGDGVGQNEKRKKWWDMNK
YHIWESMLCGYKHAYGNISENDRKMLDIPNNDDEHQFLRWFQEWTENFCTKRNELYENMV
TACNSAKCNTSNGSVDKKECTEACKNYSNFILIKKKEYQSLNSQYDMNYKETKAEKKESPEY
FKDKCNGECSCLSEYFKDETRWKNPYETLDDTEVKNNCMCKPPPPASNNTSDILQKTIPFGIA
LALGSIAFLFMKKKPKTPVDLLRVLDIPKGDYGIPTPKSSNRYIPYASDRYKGKTYIYMEGDT
SGDDDKYIWDL

>FCR3 (SEQ ID NO:56) complete 2734 aa extracellular part (577 aa highlighted corr. ID1-DBL2b)

MDSTSTIANKIEEYLGAKSDDSKIDELLKADPSEVEYYRSGGDGDYLKNNICKITVNHSDSGK
YDPCEKKLPPYDDNDQWKCQQNSSDGSGKPENICVPPRRERLCTYNLENLKFDKIRDNNAFL
ADVLLTARNEGEKIVQNHPDTNSSNVCNALERSFADLADIIRGTDQWKGTNSNLEKNLKQM
FAKIRENDKVLQDKYPKDQKYTKLREAWWNANRQKVWEVITCGARSNDLLIKRGWRTSGK
SDRKKNFELCRKCGHYEKEVPTKLDYVPQFLRWLTEWIEDFYREKQNLIDDMERHREECTR
EDHKSKEGTSYCSTCKDKCKKYCECVKKWKTEWENQENKYKDLYEQNKNKTSQKNTSRY
DDYVKDFFEKLEANYSSLE**NYIKGDPYFAEYATKLSFILNPSDANNPSGETANHNDEACNC**
NESGISSVGQAQTSGPSSNKTCITHSSIKTNKKKECKDVKLGVRENDKDLKICVIEDTSLS
GVDNCCCQDLLGILQENCSDNKRGSSSNDSCDNKNQDECQKKLEKVFASLTNGYKCDK
CKSGTSRSKKKWIWKKSSGNEEGLQEEYANTIGLPPRTQSLYLGNLPKLENVCEDVKDI
NFDTKEKFLAGCLIVSFHEGKNLKKRYPQNKNSGNKENLCKALEYSFADYGDLIKGTSI
WDNEYTKDLELNLQNNFGKLFGKYIKKNNTAEQDTSYSSLDELRESWWNTNKKYIWT
AMKHGAEMNITTCNADGSVTGSGSSCDDIPTIDLIPQYLRFLQEWVENFCEQRQAKVKD
VITNCKSCKESGNKCKTECKTKCKDECEKYKKFIEACGTAGGGIGTAGSPWSKRWDQI
YKRYSKHIEDAKRNRKAGTKNCGTSSTTNAAASTDENKCVQSDIDSFFKHLIDIGLTTPS
SYLSNVLDDNICGADKAPWTTYTTYTTTEKCNKERDKSKSQSSDTLVVVNVPSPLGNTP
YRYKYACQCKIPTNEETCDDRKEYMNQWSCGSARTMKRGYKNDNYELCKYNGVDVKPTT
VRSNSSKLDGNDVTFFNLFEQWNKEIQYQIEQYMTNANISCIDEKEVLDSVSDEGTPKVRGG
YEDGRNNNTDQGTNCKEKCKCYKLWIEKINDQWGKQKDNYNKFRSKQIYDANKGSQNKK
VVSLSNFLFFSCWEEYIQKYFNGDWSKIKNIGSDTFEFLIKKCGNNSAHGEEIFNEKLKNAEK
KCKENESTDTNINKSETSCDLNATNYIRGCQSKTYDGKIFPGKGGEKQWICKDTIIHGDTNGA
CIPPRTQNLCVGELWDKSYGGRSNIKNDTKELLKEKIKNAIHKETELLYEYHDTGTAIISKND
KKGQKGKNDPNGLPKGFCHAVQRSFIDYKNMILGTSVNIYEHIGKLQEDIKKIIEKGTPQQKD
KIGGVGSSTENVNAWWKGIEREMWDAVRCAITKINKKNNNSIFNGDECGVSPPTGNDEDQS
VSWFKEWGEQFCIERLRYEQNIREACTINGKNEKKCINSKSGQGDKIQGACKRKCEKYKKYI
SEKKQEWDKQKTKYENKYVGKSASDLLKENYPECISANFDFIFNDNIEYKTYYPYGDYSSICS
CEQVKYYKYNNAEKKNNKSLCYEKDNDMTWSKKYIKKLENGRSLEGVYVPPRRQQLCLYE
LFPIIIKNEEGMEKAKEELLETLQIVAEREAYYLWKQYNPTGKGIDDANKKACCAIRGSFYDL
EDIIKGNDLVHDEYTKYIDSKLNEIFGSSDTNDIDTKRARTDWWENETITNGTDRKTIRQLVW
DAMQSGVRYAVEEKNENFPLCMGVEHIGIAKPQFIRWLEEWTNEFCEKYTKYFEDMKSKCD
PPKRADTCGDNSNIECKKACANYTNWLNPKRIEWNGMSNYYNKIYRKSNKESEGGKDYSMI
MAPTVIDYLNKRCHGEINGNYICCSCKNIGAYNTTSGTVNKKLQKKETECEEEKGPLDLMNE
VLNKMDKKYSAHKMKCTEVYLEHVEEQLNEIDNAIKDYKLYPLDRCFDDQTKMKVCDLIA
DAIGCKDKTKLDELDEWNDMDLRGTYNKHKGVLIPPRRRQLCFSRIVRGPANLRSLNEFKEE

ILKGAQSEGKFLGNYYKEHKDKEKALEAMKNSFYDYEDIIKGTDMLTNIEFKDIKIKLDRLLE
KETNNTKKAEDWWKTNKKSIWNAMLCGYKKSGNKIIDPSWCTIPTTETPPQFLRWIKEWGT
NVCIQKQEHKEYVKSKCSNVTNLGAQASESNNCTSEIKKYQEWSRKRSIRWETISKRYKKYK
RMDILKDVKEPDANTYLREHCSKCPCGFNDMEEMNNNEDNEKEAFKQIKEQVKIPAELEDVI
YRIKHHEYDKGNDYICNKYKNIHDRMKKNNGNFVTDNFVKKSWEISNGVLIPPRRKNLFLYI
DPSKICEYKKDPKLFKDFIYWSAFTEVERLKKAYGGARAKVVHAMKYSFTDIGSIIKGDDMM
EKNSSDKIGKILGDTDGQNEKRKKWWDMNKYHIWESMLCGYREAEGDTETNENCRFPDIES
VPQFLRWFQEWSENFCDRRQKLYDKLNSECISAECTNGSVDNSKCTHACVNYKNYILTKKT
EYEIQTNKYDNEFKNKNSNDKDAPDYLKEKCNDNKCECLNKHIDDKNKTWKNPYETLEDTF
KSKCDCPKPLPSPIKPDDLPPQADEPFDPTILQTTIPFGIALALGSIAFLFMKVIYIYIYVCCICMY
VCMYVCMYVCMYVCMYVCMHVCMLCVYVIYVFKICIYIEKEKRKK

>BPTI, protease inhibitor (SEQ ID NO:57)

RPDFCLEPPYTGPCKARIIRYFYNAKAGLCQTFVYGGCRAKRNNFKSAEDCMRTCGGA