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(71) Applicant: **NATIONAL UNIVERSITY OF SINGAPORE** [SG/SG]; 21 Lower Kent Ridge Road, Singapore 119077 (SG).

(72) Inventors: **MORINAKA, Brandon Isamu**; c/o National University of Singapore, Faculty of Science / Dept of Pharmacy, 21 Lower Kent Ridge Road, Singapore 119077 (SG). **SUGIYAMA, Ryosuke**; c/o National University of Singapore, Faculty of Science / Dept of Pharmacy, 21 Lower Kent Ridge Road, Singapore 119077 (SG). **YAO, Ziwei**; c/o National University of Singapore, Faculty of Science / Dept of Pharmacy, 21 Lower Kent Ridge Road, Singapore 119077 (SG). **EE, Pui Lai Rachel**; c/o National University of Singapore, Faculty of Science / Dept of Pharmacy, 21 Lower Kent Ridge Road, Singapore 119077 (SG). **TRAM, Dai Thien Nhan**; c/o National University of Singapore, Faculty of Science / Dept of Pharmacy, 21 Lower Kent Ridge Road, Singapore 119077 (SG). **MORISHITA, Yohei**; c/o National University of Singapore, Faculty of Science / Dept of Pharmacy, 21 Lower Kent Ridge Road, Singapore 119077 (SG). **PHAN, Chin-Soon**; c/o National University of Singapore, Faculty of Science / Dept of Pharmacy, 21 Lower Kent Ridge Road, Singapore 119077 (SG). **LIM, Joel**; c/o National University of Singapore, Faculty of Science / Dept of Pharmacy, 21 Lower Kent Ridge Road, Singapore 119077 (SG).

(74) Agent: **DAVIES COLLISON CAVE ASIA PTE. LTD.**; 10 Collyer Quay #07-01, Ocean Financial Centre, Singapore 049315 (SG).

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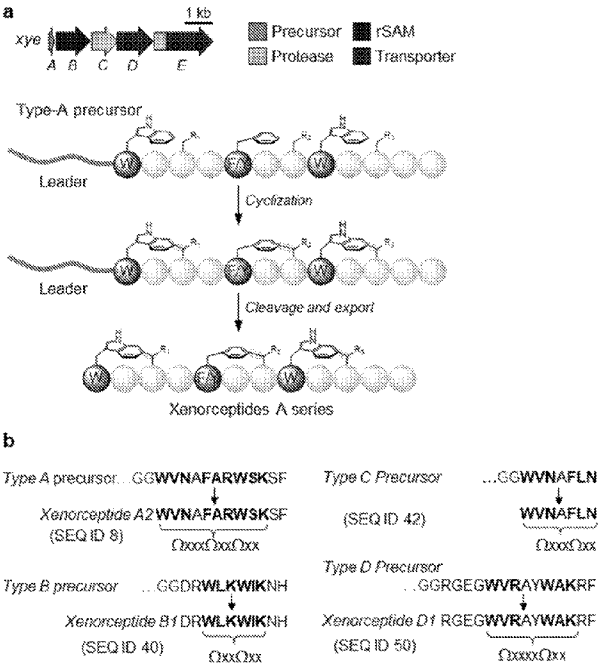

Figure 1

(57) Abstract: The present disclosure concerns a polypeptide comprising a first three residue motif (from a N-terminus) and a second three residue motif, the first and second three residue motif optionally separated by 1 to 3 amino acid residue, and at least two C-terminus residues. The three residue motif is each represented by X_1-X_2-X_3. Each X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine, an unnatural aromatic amino acid residue or a derivative thereof. Each X_2 and X_3 are independently any amino acid residue. X_1 and X_3 in each motif are connected to form a cyclophane moiety. At least one of the two C-terminus residues is an aromatic residue. The present disclosure also concerns a method of producing the polypeptide.

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Peptides with Antimicrobial Properties

Technical Field

The present invention relates, in general terms, to peptides with antimicrobial properties and the methods of synthesising the peptides thereof.

Background

The CDC and WHO classify Carbapenem-resistant Enterobacteriaceae (CRE) which include the Gram-negative bacteria *Klebsiella pneumoniae* and *Escherichia coli* as two of the highest priority pathogens for which new antibiotics are urgently needed. CRE are an immediate threat because of their resistance to any carbapenem and their 50% increase over the last 5 years. Extended-spectrum β-lactamase–producing Enterobacterales (ESBL-E) account for a greater number of cases and more deaths compared to CRE but may still be treated with selected carbapenem antibiotics. The increased use of carbapenems, along with transmission of various resistance mechanisms have likely contributed to the rise in CRE. Both CRE and ESBL-E can lead to severe and deadly infections in hospital and nursing home patients via pneumonia, bloodstream infections, urinary tract infections, wound infections, and meningitis. New antibiotics able to treat both types of infections would reduce the mortality rate and decrease the spread of resistance mechanisms.

Ribosomally synthesized and posttranslationally modified peptides (RiPPs) are a rapidly growing family of natural products with potential antibiotic activities against a broad range of pathogens. RiPPs may be biosynthesized from a ribosomally synthesized precursor, posttranslationally modified, cleaved, then exported to give the mature RiPP. For example, RiPP pathways involving radical *S*-adenosylmethionine (rSAM) enzymes in their biosynthesis are of particular interest due to their ability to catalyze distinct chemically-demanding reactions leading to unique and bioactive RiPP natural products. The structural diversity and antibiotic activities are demonstrated by several RiPP families including lasso peptides, plantazolicins, lanthipeptides, thiopeptides, and sactipeptides. RiPP biosynthetic gene clusters (BGCs) are attractive for genome mining and synthetic biology due to their compact size and ease of genetic manipulation. For chemically-guided discovery, RiPP pathways are particularly appealing because a single

posttranslational modifying enzyme can create unique, structurally complex, and bioactive peptides. Since RiPP biosynthesis is determined by a logic rather than genetically tractable features, their true number and diversity remains enigmatic and a promising source for new peptide scaffolds and antibiotics.

It would be desirable to overcome or ameliorate at least one of the above-described problems.

Summary

The present invention provides a polypeptide comprising:

a) a first three residue motif (from a N-terminus) and a second three residue motif, the first and second three residue motif optionally separated by 1 to 3 amino acid residue; and

b) at least two C-terminus residues;

wherein the three residue motif is each represented by X_1-X_2-X_3;

wherein each X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine, an unnatural aromatic amino acid residue or a derivative thereof;

wherein each X_2 and X_3 are independently any amino acid residue;

wherein X_1 and X_3 in each motif are connected to form a cyclophane moiety;

wherein at least one of the two C-terminus residues is an aromatic residue.

In some embodiments, the first and second three residue motifs are separated by 1 to 3 amino acid residue.

In some embodiments, the first three residue motif is not fused with the second three residue motif via the cyclophane moieties.

In some embodiments, the first X_1 is a residue selected from tryptophan, phenylalanine or a derivative thereof and the second X_1 is a residue selected from phenylalanine, tyrosine or a derivative thereof.

In some embodiments, X_2 is an amino acid residue, the amino acid independently selected from I, G, E, Y, V, L, A, D, S, T, N or Q.

In some embodiments, X_3 is an amino acid residue, the amino acid independently selected from N, R, S, D, Q or K.

In some embodiments, at least one of the two C-terminus residues is a polar and/or basic residue.

In some embodiments, at least one of the two C-terminus residues is an aromatic residue.

In some embodiments, the polypeptide comprises a third three residue motif.

In some embodiments, when the polypeptide comprises a third three residue motif, X_3 of the first motif and X_1 of the second motif are separated by 1 amino acid residue, and X_3 of the second motif and X_1 of the third motif are covalently bonded to each other via an amide bond.

In some embodiments, the third X_1 is a residue independently selected from tryptophan, phenylalanine or a derivative thereof.

In some embodiments, the polypeptide is represented by Formula (I):

$$X_1\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad (I)$$

wherein each X_1 is an amino acid residue, the amino acid independently selected from tryptophan, phenylalanine or a derivative thereof;
wherein each X_2 is an amino acid residue, the amino acid independently selected from leucine, isoleucine, valine, alanine, proline, serine, lysine, asparagine, phenylalanine, aspartic acid or a derivative thereof;
wherein each X_3 is an amino acid residue, the amino acid independently selected from lysine, glutamine, asparagine, arginine or a derivative thereof;
wherein X_n is an amide bond or 1 to 3 amino acid residue; and
wherein X_m is at least two C-terminus residues.

In some embodiments, the polypeptide is represented by Formula (II):

$$X_1\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad (II)$$

wherein each X_1 is an amino acid residue, the amino acid independently selected from tryptophan, phenylalanine, tyrosine or a derivative thereof;

wherein each X_2 is an amino acid residue, the amino acid independently selected from valine, isoleucine, phenylalanine, tryptophan, alanine, leucine, glycine, serine, proline, threonine, aspartic acid, asparagine, glutamic acid, arginine or a derivative thereof;
wherein each X_3 is an amino acid residue, the amino acid independently selected from arginine, lysine, asparagine or a derivative thereof;
wherein X_n is an amide bond or 1 to 3 amino acid residue; and
wherein X_m is at least two C-terminus residues.

In some embodiments, X_1 and X_3 in the second motif are connected via phenylene to form a cyclophane moiety.

In some embodiments, the polypeptide is represented by Formula (Ia), (IIa), (Id) or (IId):

$$W\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad (Ia)$$
$$W\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad (IIa)$$
$$F\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad (Id)$$
$$F\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad (IId).$$

In some embodiments, when X_1 is W, X_1 is connected to X_3 via a 3,6 or 3,7 substituted indolylene moiety. It was found that the 3,6 or 3,7 substitution is advantageous for providing an antibacterial effect.

In some embodiments, the polypeptide is represented by Formula (Ib), (IIb), (Ie) or (IIe):

$$W\text{-}X_2\text{-}X_3\text{-}X_n\text{-}F/Y\text{-}X_2\text{-}X_3\text{-}X_m \quad (Ib)$$
$$W\text{-}X_2\text{-}X_3\text{-}X_n\text{-}F/Y\text{-}X_2\text{-}X_3\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad (IIb)$$
$$F\text{-}X_2\text{-}X_3\text{-}X_n\text{-}W\text{-}X_2\text{-}X_3\text{-}X_m \quad (Ie)$$
$$F\text{-}X_2\text{-}X_3\text{-}X_n\text{-}W\text{-}X_2\text{-}X_3\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad (IIe).$$

In some embodiments, when X_1 is F or Y, X_1 is connected to X_3 via a 1,3 or 1,4 disubstituted phenylene moiety. In some embodiments, when X_1 is F or Y, X_1 is connected to X_3 via a 1,3 disubstituted phenylene moiety.

In some embodiments, the polypeptide is represented by Formula (IIc):

$$W\text{-}X_2\text{-}X_3\text{-}X_n\text{-}F/Y\text{-}X_2\text{-}X_3\text{-}W\text{-}X_2\text{-}X_3\text{-}X_m \quad (IIc).$$

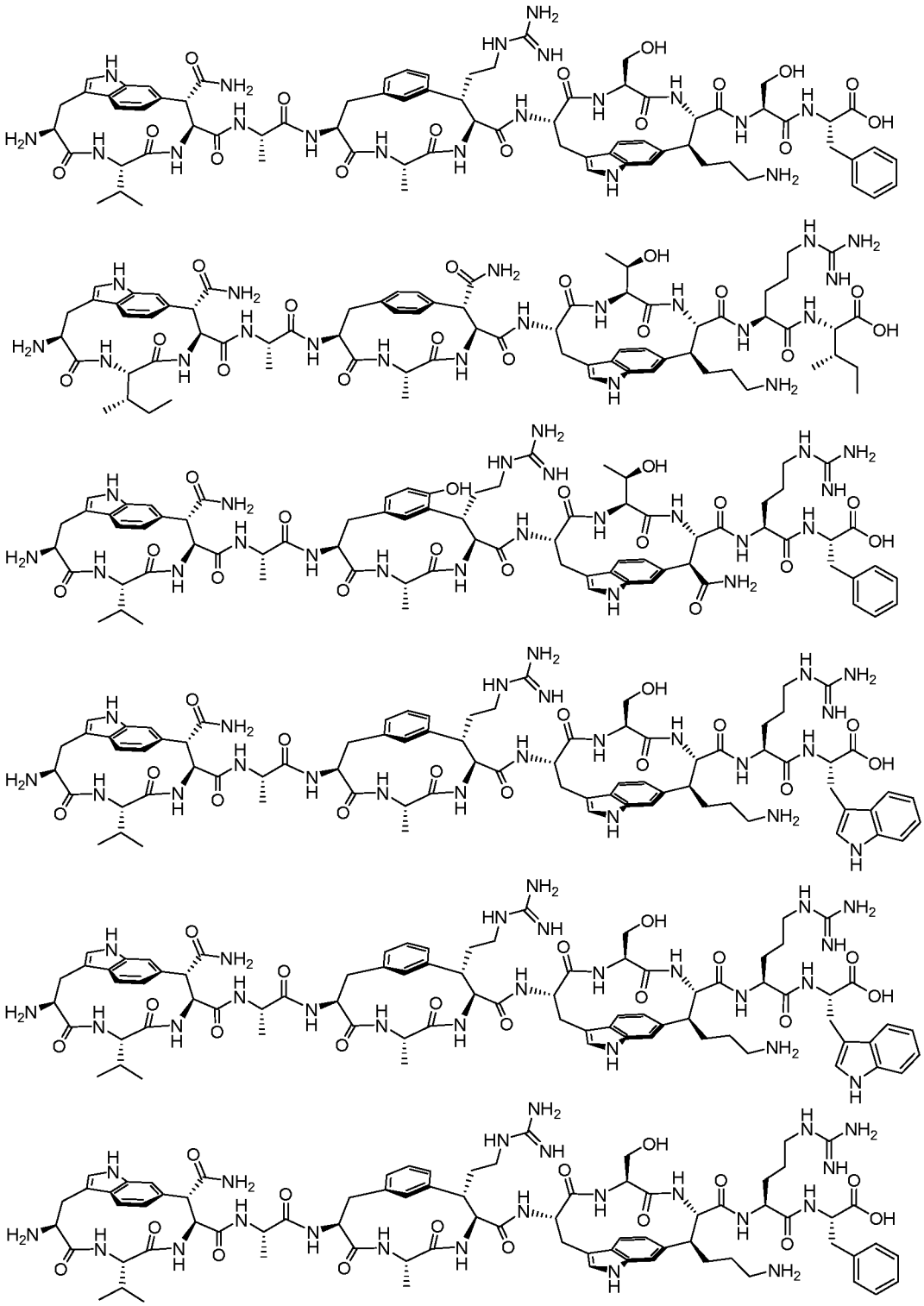
In some embodiments, the polypeptide is selected from:

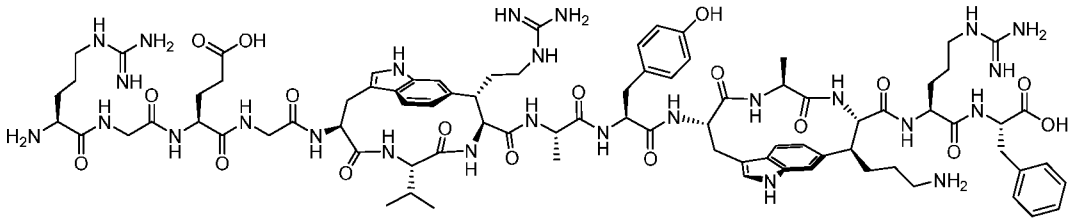
WVNA**F**AN**W**TKRF (SEQ ID 19)
WVNA**F**AN**W**PKRF (SEQ ID 17)
WINA**F**AN**W**TKRI (SEQ ID 13)
WWRA**Y**AR**W**RRSF (SEQ ID 37)
WVNA**F**AR**W**GKSF (SEQ ID 4)
G**W**FRA**Y**LR**W**SRSF (SEQ ID 36)
WVNA**Y**AR**W**TNRF (SEQ ID 25)
WVNA**F**AK**W**TKRI (SEQ ID 14)
WVNA**Y**AR**W**TKRF (SEQ ID 26)
WVNV**F**AR**W**DKQI (SEQ ID 22)
WVNF**F**AK**F**TKSF (SEQ ID 15)
WVNA**F**AR**W**SRRW (SEQ ID 30)
WVNA**F**AR**W**SKSF (SEQ ID 8)
WVNV**F**AR**W**SRRW (SEQ ID 34)
AG**W**IRA**F**AN**W**SRSF (SEQ ID 35)
WVNA**F**AR**W**DKKF (SEQ ID 23)
WVNA**F**AR**F**TKRF (SEQ ID 20)
WVNV**F**AR**W**DKAI (SEQ ID 10)
WLNV**F**VR**W**DRAI (SEQ ID 24)
WINV**F**AR**W**NRAI (SEQ ID 21)
WINA**F**GN**W**ERAFH (SEQ ID 32)
WVNA**F**AN**W**SKSF (SEQ ID 3)
WVNA**F**AN**W**SKAL (SEQ ID 1)
WVNA**F**GN**W**SKSL (SEQ ID 2)
WVNA**F**LN**W**SRSF (SEQ ID 16)
WVNA**F**LR**W**GKSF (SEQ ID 12)
WINA**F**AR**W**GRAF (SEQ ID 7)
AG**W**IKV**F**GN**W**SRSF (SEQ ID 33)
WVNA**F**VN**W**TKSF (SEQ ID 9)
WVNA**F**LN**W**PRSF (SEQ ID 18)
AG**W**IKA**F**GN**W**SRSF (SEQ ID 29)
WVNA**F**VN**W**PKSF (SEQ ID 6)
AG**W**INA**F**AN**W**TKSF (SEQ ID 28)

AG**W**INA**F**AN**W**TRSF (SEQ ID 31)
AG**W**INA**F**GN**W**TKSF (SEQ ID 27)
WVNA**F**AR**W**GRAF (SEQ ID 5)
WVNA**F**AR**W**SKRW (SEQ ID 38)
WVNA**F**AR**W**SKRF (SEQ ID 39)
RGEG**W**VRAY**W**AKRF (SEQ ID 50)
KPGEG**W**VNFT**W**NKSF (SEQ ID 52)
KSEAAGG**W**VNFQ**W**KNSW (SEQ ID 46)
AGNDG**W**VKFG**W**KKKF (SEQ ID 49)
ASTAET**W**FKLD**W**KKSF (SEQ ID 54)
DGR**W**LQ**W**IKNH (SEQ ID 41)
GDR**W**LK**W**IKNH (SEQ ID 40)
VGG**F**ANAT**W**SKSF (SEQ ID 44)
VGG**F**ANAS**W**PKSF (SEQ ID 43)
VGG**F**ANAT**W**PKSF (SEQ ID 45)
NA**F**VNAT**W**SRAM (SEQ ID 59)
NV**F**VNATWSRAM (SEQ ID 47)
NV**F**VNAT**W**SRAI (SEQ ID 60)
SSDDDGI**F**FKTT**W**DRR (SEQ ID 55)

.

In some embodiments, the polypeptide is selected from:





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In some embodiments, the polypeptide is an isolated polypeptide.

In some embodiments, the polypeptide is characterised by an antibacterial activity. In some embodiments, the polypeptide is characterised by an antibacterial activity against Gram-negative bacteria. In some embodiments, the polypeptide is characterised by an antibacterial activity against drug-resistant bacteria.

In some embodiments, the polypeptide is characterised by a minimal inhibitory concentration (MIC) of about 2 μg/mL to about 10 μg/mL.

The present invention also provides a composition comprising a polypeptide as disclosed herein.

The present invention also provides a method of producing a polypeptide in a host cell, the method comprising:
a) introducing to the host cell one or more nucleic acid molecules, the nucleic acid molecules configured to express a precursor polypeptide (A), a rSAM/SPASM maturase (B), a protease (C), a transporter (D) and a protease/transporter (E);
wherein the precursor polypeptide comprises a first three residue motif (from a N-terminus) and a second three residue motif, the first and second three residue motif optionally separated by 1 to 3 amino acid residue, and at least two C-terminus residues;
wherein the three residue motif is each represented by X_1-X_2-X_3;
wherein each X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine, an unnatural aromatic amino acid residue or a derivative thereof;
wherein each X_2 and X_3 are independently any amino acid residue;
wherein at least one of the two C-terminus residues is an aromatic residue;
wherein the rSAM/SPASM maturase is capable of modifying the precursor polypeptide in the host cell to form a modified precursor polypeptide with a cyclophane moiety

connecting the X_1 and X_3 residues in each motif;
wherein the protease, transporter and protease/transporter are capable of cleaving the modified precursor polypeptide from the rSAM/SPASM maturase to form a cleaved modified polypeptide and exporting the cleaved modified polypeptide out from the host cell.

In some embodiments, at least the nucleic acid molecule configured to express A is derived from a Xye maturase system.

In some embodiments, the nucleic acid molecules configured to express A and B are from one Xye species and the nucleic acid molecules configured to express C, D and E are from another Xye species.

In some embodiments, at least the nucleic acid molecules configured to express C, D and E are fused.

In some embodiments, the nucleic acid molecules configured to express A and B are fused.

In some embodiments, the nucleic acid molecules configured to express B, C, D and E are fused.

In some embodiments, the nucleic acid molecules configured to express A, B, C, D and E are fused.

In some embodiments, the nucleic acid molecule configured to express A is at least 70% identical to and derived from a bacterial species selected from *Serratia marcescens (smc)*, *Erwinia toletana (etc)*, *Photorhabdus australis (pac), Xenorhabdus nematophila (xnc), Xenorhabdus griffiniae VH1 (xgc), Pandoraea sp. PE-S2R-1 (psc), Pandoraea oxalativorans DSM 23570 (poc), Photorhabdus heterorhabditis Q614 (phc), Kosakonia cowanii pasteuri (kcc2 and kcc1), Bordetella bronchialis AU17976 (bbc)* and *Photorhabdus laumondii BOJ-47 (plc).*

In some embodiments, the nucleic acid molecules configured to express C, D and E are at least 70% identical to and derived from *Xenorhabdus nematophila (xnc).*

In some embodiments, the rSAM/SPASM maturase has an amino acid sequence that is at least 70% identical to one of the following:

XncB:

MTTSKSEKIKHLEIILKISERCNINCSYCYVFNMGNSLATDSPPVISLDNVLALRGFFERSAAENEI
EVIQVDFHGGEPLMMKKDRFDQMCDILRQGDYSGSRLELALQTNGILIDDEWISLFEKHKVHASI
SIDGPKHINDRYRLDRKGKSTYEGTIHGLRMLQNAWKQGRLPGEPGILSVANPTANGAEIYHHFA
NVLKCQHFDFLIPDAHHDDDIDGIGIGRFMNEALDAWFADGRSEIFVRIFNTYLGTMLSNQFYRV
IGMSANVESAYAFTVTADGLLRIDDTLRSTSDEIFNAIGHLSELSLSGVLNSPNVKEYLSLNSELPS
DCADCVWNKICHGGRLVNRFSRANRFNNKTVFCSSMRLFLSRAASHLITAGIDEETIMKNIQK
(SEQ ID NO: 61)

YkcB:

MEVITGSEGRVMLNLLIEKNIRHLEIILKISERCNINCDYCYVFNKGNSAADDSPARLSNKNIHHLV
CFLQRACQEYKIGTVQIDFHGGEPLLMKKENFTDMCIQLISGNYCGSNIRLALQTNATLIDNEWIA
IFEKYSVNVSISIDGPKHINDRHRLDTKGRSTYESTVRGLRILQNAYQQGRLPSDPGILCVTNAQA
NGAEIYRHFVDELGVYSFDFLIPDDSYKDAHPDAVGIGRFLNEALDEWVKDNNAKIFVRLFQTHIA
SLLGQKNSGVLGHTPNITGVYALTVSSDGFVRVDDTLRSTSDRMFNPIGHLSEVNLSNVFASPQF
QEYSSIGQSLPTECEGCIWENICAGGRIVNRFSTEDRFKHKSIYCYSMRTFLSRSSAHLLNMGIKE
ERIMAAIRA (SEQ ID NO: 62)

EtcB:

MTQLKGEKIKHLEIILKISERCNINCTYCYVFNMGNTLATDSTPVISLDNVYALRGFFERSAAENDI
EVIQVDFHGGEPLMMKKDRFDRMCQILLQGNYRSSKFELALQTNGILIDDEWIALFEKHQVHASI
SVDGPKHINDRHRLDRKGKSTYEGTITGLRLLQNAWQQGRLPGEPGILSVANANANGAEIYRHF
ADTLQCQRFDFLIPDDHHDDSPDGEGVGRFLNEALDAWFADGRPEIFIRIFNTYLGTMLNSQFNR
VLGMSANVESAYAFTVTADGMLRIDDTLRSTSDEIFNAVGHVSELSLARVLETSCVKEYLALSSNL
PTVCAECVWNNICHGGRLVNRFSRTNRFNNKTVFCKSMRLFLSRAASHLMASGVDEKEIMKNIQ
K (SEQ ID NO: 63)

MscB

MAPGPARAALTEFVLKVHARCDLACDHCYVYEHADQSWRRRPVRMTPEVLRTAAGRIAEHAAAH
DLPDVTVILHGGEPLLLGAERLGEVLADLRRVIDPVTRLRLGMQTNGVLLSERLCDLLAEHDVAVG
VSLDGDRAANDRHRRFRSGAGSYDQVLRAIGLLRRPAYRRIYSGLLCTVDVRNDPIAVYESLLTQ
EPPRIDFLLPHATWDDPPWRPAGGGTAYAGWLRAVYDRWLADGRPVSVRLFDSLLSTAAGGPS

GTEWLGLDPVDLAVVETDGEWEQADSLKTAYDGAPATGMTVFSHAADDVAASPLLARRRSGRA
GLSDECRRCPVVDQCGGGLFAHRYGAGHFDHPSVYCADLKELIVHVNENPPAPVRLDAGLPDDF
IDRLAALTGDRVAIGRLVEAQIAIVRALLAEVADRLPAGGAGADGWEALTALDRSAPESVARIAAH
PYVRAWAVDCLAGSGTGARQGPDYLSALAVAAALDAGTPVRLDVPVRSGRLHLPTVGTVLLPEV
GDGAARVETGPGSLRVAAGDVTVAIRPGTPGDAPRWWPTRVLAAPDVSVLLEDGDPHRDCHRL
PAGDRLDDAGAARWAETFAAAWQVIRDEVPGHAEELRAGLRAVVPLRRSGAGVSEASTARQAF
GGVAATETDAGSLAVLLVHEFQHSKMNALLDICDLVDGTRPIDITVGWRPDPRPAEAVLHGIYAH
AAVADIWRIRADRQVDGAQAVYRRYRDWTAEAIGALQRADALTPAGSRLVRQVARSMSGWPS
(SEQ ID NO: 64)

OscB:
MINPTLLNPEKIDISKFGPINLVVIQATSFCNLNCDYCYLPNRDLKNTLSLDLIEPIFKNIFNSPFVG
DEFTICWHAGEPLAVPISFYESAFQLIQAADQKYNQKQAKIWHSVQTNATYINQKWCDFIQEHNI
CVGVSLDGPEFIHDAHRQTRKGTGSHAQTMRGISFLQKNNIPFYVISVVTQDSLNYADEIFNFFR
ENGIYDVGFNLEEIEGVNQSSTLEAVGTSEKYRAFMQRFWELTSEVQGEFNLREFEAICGLIYSNT
RLTQTDMNNPFVLINIDYQGNFSTFDPELLSVNIKPYGNFILGNVLTDSFESVCDTEKFQKIYTDM
QEGIKLCRETCEYFGVCGGGAGSNKYWENGTFACSETMACRYRIKVVTDIILDKLENSLGLVENC
(SEQ ID NO: 65)
LscB:
MTISKMNLPVQTDNFRASSTLDLSAFGPINLVVIQSTSFCNLNCDYCYLRDRQSKNRLSLDLIEPIL
KTVLTSPFVGCDFTILWHAGEPLAMPISFYDSATALIREAERQYKTQPIQIFQSIQTNATLINQAWC
DCFRRNEIYVGVSLDGPAFLHDAHRQTYKGTGTHAATMRGISLLQKNEIPFNVICVLTQDSLDYP
DEIFNFFRSNRITEVGFNMEEAEGVHQHSTLDQQGTEERYRAFMQRFWDLTVQAKGEFKLREFE
TICTLAYTGDRLGYTDMNQPFVIVNFDHQGNFSTFDPELLSFKIKEYGDFVLGNVLHNTLESVCQT
EKFQKIYQDMAAGVVQCRQSCEYFGLCGGGAGSNKYWENGTFNCTETKACRYRIKVIADIVLEG
LENSLELANSIS (SEQ ID NO: 66)

GscB
MSIVTSKPVINFKNTANFGPISLIIIQPNSFCNLDCDYCYLPDRHLQNKLSLDLIDPIFKSIFTSPFLG
CDFGVCWHAGEPLTMPVSFYKSAFQLIEEANTKYNKSEYSFYHSYQTNGTLINQGWCDLWQEYP
VHVGVSIDGPAFLHDVHRKNRKGGNSHDLTMRGIRYLQKNNIPYNTISVITEESLNYPDEMFNFF
AENEIYDLAFNMEETEGVNELTSLNGIEIEHKYSQFIKRFWQLVTESKLPFIVREFEILISLIYSGNR
LTNTDMNKPFVIVNFDYQGNFSTFDPELLSVKTDKYGDFIFGNVLKDSLESICETEKFKTIYKDIND
GVKLCSDNCSYFGICGGGAGSNKYWENGTFASMETQACRYRIKILTDVLVSTIENSLGL (SEQ
ID NO: 67)

MscB-375
MAPGPARAALTEFVLKVHARCDLACDHCYVYEHADQSWRRRPVRMTPEVLRTAAGRIAEHAAAH
DLPDVTVILHGGEPLLLGAERLGEVLADLRRVIDPVTRLRLGMQTNGVLLSERLCDLLAEHDVAVG
VSLDGDRAANDRHRRFRSGAGSYDQVLRAIGLLRRPAYRRIYSGLLCTVDVRNDPIAVYESLLTQ
EPPRIDFLLPHATWDDPPWRPAGGGTAYAGWLRAVYDRWLADGRPVSVRLFDSLLSTAAGGPS
GTEWLGLDPVDLAVVETDGEWEQADSLKTAYDGAPATGMTVFSHAADDVAASPLLARRRSGRA
GLSDECRRCPVVDQCGGGLFAHRYGAGHFDHPSVYCADLKELIVHVNENPPAPV (SEQ ID NO: 68).

In some embodiments, the rSAM/SPASM maturase is characterised by a rSAM domain and a SPASM domain;
wherein the rSAM domain is selected from CNINCSYC (SEQ ID NO: 69), CNINCDYCYVFNK (SEQ ID NO: 213), CNINCTYC (SEQ ID NO: 215), CDLACDHC (SEQ ID NO: 217), CNLNCDYC (SEQ ID NO: 219), CNLNCDYC (SEQ ID NO: 221), and CNLDCDYC (SEQ ID NO: 223); and
wherein the SPASM domain is selected from CADCVWNKIC (SEQ ID NO: 70), CEGCIWENIC (SEQ ID NO: 214), CAECVWNNIC (SEQ ID NO: 216), CRRCPVVDQC (SEQ ID NO: 218), CRETCEYFGVC (SEQ ID NO: 220), CRQSCEYFGLC (SEQ ID NO: 222), and CSDNCSYFGIC (SEQ ID NO: 224).

In some embodiments, the nucleic acid molecules are introduced into the host cell via a pET28a(+) vector, pCDFduet-1 vector, pACYCDuet-1 vector, pETDuet-1 vector, pCOLADuet-1 vector, pRSFDuet-1 vector, pBAD vector, or a combination thereof.

In some embodiments, the host cell is E. coli NiCo21(DE3), BL21(DE3), BL21-AI, BL21 Star™ (DE3) pLysS, Rosetta™(DE3), or a combination thereof.

The present invention also provides a method of producing a polypeptide, the method comprising:
a) expressing a precursor polypeptide and a rSAM/SPASM maturase;
wherein the precursor polypeptide comprises a first three residue motif (from a N-terminus) and a second three residue motif, the first and second three residue motif optionally separated by 1 to 3 amino acid residue, and at least two C-terminus residues;
wherein the three residue motif is each represented by X_1-X_2-X_3;

wherein each X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine, an unnatural aromatic amino acid residue or a derivative thereof;
wherein each X_2 and X_3 are independently any amino acid residue;
wherein at least one of the two C-terminus residues is an aromatic residue;
wherein the rSAM/SPASM maturase is capable of modifying the precursor polypeptide to form a polypeptide with a cyclophane moiety connecting the X_1 and X_3 residues in each motif.

The present invention also provides a method of synthesising a polypeptide as disclosed herein, the method comprising:
(a) coupling a pre-sequence peptide to a support, wherein said pre-sequence peptide comprises amino acid residues having side chain functionalities which are, if necessary, protected during the synthesis;
(b) coupling one or more N-protected amino acids to the N-terminus of the pre-sequence peptide to form a precursor polypeptide, wherein each coupling is performed in stepwise fashion and under conditions in which each of the amino acids of the target peptide is coupled and subsequently N-deprotected;
c) cleaving said precursor polypeptide from the support; and
d) synthetically or enzymatically connecting the X_1 and X_3 in each motif to form a cyclophane moiety.

The present invention also provides a method of modifying a precursor polypeptide, the precursor polypeptide comprising:
a) a first three residue motif (from a N-terminus) and a second three residue motif, the first and second three residue motif optionally separated by 1 to 3 amino acid residue; and
b) at least two C-terminus residues;
wherein the three residue motif is each represented by X_1-X_2-X_3;
wherein each X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine, an unnatural aromatic amino acid residue or a derivative thereof;
wherein each X_2 and X_3 are independently any amino acid residue; and
wherein at least one of the two C-terminus residues is an aromatic residue;
the method comprising:
enzymatically connecting the X_1 and X_3 residues in each motif to form a cyclophane moiety.

In some embodiments, the enzyme is rSAM/SPASM maturase.

The present invention also provides a method of treating a bacterial infection, comprising administering an effective amount of a polypeptide as disclosed herein to subject in need thereof.

In some embodiments, the bacterial infection is a Gram-negative bacterial infection. In some embodiments, the bacterial infection is characterised by a drug-resistance.

In some embodiments, the bacterial infection is caused by a Gram-negative bacteria selected from *Escherichiacoli, Pseudomonas aeruginosa, Candidatus Liberibacter, Agrobacterium tumefaciens, Acinetobactor baumannii, Moraxella catarrhalis, Citrobacter di versus, Enterobacter aerogenes, Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhimurium, Neisseria meningitidis, Serratia marcescens, Shigella sonnei, Shigella boydii, Neisseria gonorrhoeae, Acinetobacter baumannii, Salmonella enteriditis, Fusobacterium nucleatum, Veillonella parvula, Actinobacillus actinomycetemcomitans, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Helicobacter pylori, Francisella tularensis, Yersinia pestis, Vibrio cholera, Morganella morganii, Edwardsiella tarda, Campylobacter jejuni, Haemophilus influenza, Enterobacter cloacae*, or a combination thereof.

Brief description of the drawings

Embodiments of the present invention will now be described, by way of non-limiting example, with reference to the drawings in which:

Figure 1. Biosynthesis and types of Xenorceptides.
Figure 2. Chemically-guided workflow for RiPP antibiotic discovery (GEnSyBER-A). Genomic enzymology identifies sequence-function space of a RiPP family based on posttranslational modifying enzyme. Synthetic biology provides the targeted natural products. Structure elucidation unveils the chemical structure. Antibacterial assays reveal any bioactivity against pathogens of interest. Sequence similarity network containing SPASM/Twitch proteins (Alignment score = 45) taken from RadicalSAM.org.

Figure 3. Production of Xenorceptides. **a**, Coexpression of His_6-SmcA+SmcB. **b**, Production of natural product using a 2-vector system, His_6-AB/pET28 + CDE/pCDFDuet-1. EICs show cleaved leader (left) and natural product (right) detected only when coexpressed with SmcCDE. HR-MS for **2** is shown. **c**, Summary of constructs used to produce **2**-**4**. Coexpressions with XncCDE provide increased production of natural product.

Figure 4. Source BGCs/strains, structures, and NOESY correlations. **a**, Structures of xenorceptide A1 (**1**), xenorceptide A2 (**2**), xenorceptide A3 (**3**), and xenorceptide A4 (**4**). **b**, Key NOESY correlations used to assign the substitution and conformation of Phe- and Tyr-derived cyclophanes.

Figure 5. Biological evaluation of xenorceptide A2 (2). **a**, Time-kill kinetics of xenorceptide A2 (**2**) against *E. coli* M6 over 24 h. Colistin at 2×MIC was tested as a positive control. Black dotted lines indicate the limit of detection (50 CFU/mL). Experiments were repeated on three biologically independent samples. Data are presented as geometric mean ± SE. **b**, SEM images of *E. coli* M6 cells either untreated or after treatment with 8×MIC xenorceptide A2 (**2**) for 2 h. For each sample slide, at least five independent fields were imaged to ensure representativeness. Magnification = 20,000×. **c**, the development of resistance of *E. coli* M6 against xenorceptide A2 (**2**) was monitored using serial passage over 14 days. Experiments were repeated on three independent starting cultures.

Figure 6. Test expression of *xnc* genes. **a**, Test expression for precursor and rSAM/SPASM by coexpression of His_6-XncA+XncB. EICs show modified fragment. HR-MS for the modified fragment is shown. **b**, Coexpression using a 2-vector system, *His_6-xncAB*/pET28 + *xncCDE*/pCDFDuet-1. EICs show cleaved leader, suggesting peptidase cleaves precursor peptide.

Figure 7. xye BGCs from *Serratia marcescens*, *Erwinia toletana*, and *Photorhabdus australis*.

Figure 8. Production of xenorceptide A3. **a**, Test expression for precursor and rSAM/SPASM by coexpression of His_6-EtcA+EtcB. **b**, Production of natural product using a 2-vector system, *His_6-etcAB*/pET28 + *etcCDE*/pCDFDuet-1. EICs show cleaved leader (left) only when coexpressed with EtcCDE, while natural product is not detected (right).

Figure 9. Production of xenorceptide A4. **a**, Test expression for precursor and rSAM/SPASM by coexpression of His_6-EtcA+EtcB. **b**, Production of natural product using a 2-vector system, *His_6-pacAB*/pET28 + *pacCDE*/pCDFDuet-1. EICs show cleaved leader (left) only when coexpressed with PacCDE, while natural product is not detected (right).

Figure 10. RiPP cyclophane natural products: darobactin, dynobactin, and triceptides. a, Chemical structures for darobactin, dynobactin and xenorceptide A1 from the dar, dyn, and xnc BGCs respectively. Xenorceptide A1 is a representative xenorceptide. b, Canonical cyclophanes from each class. c, Schematic showing location of Cys residues corresponding to three Fe-S clusters in DarE, DynA, and 3-CyFE maturases. The CX3CX2C motif for the rSAM Fe-S cluster and the CX2-3CX4-6C motif with additional Cys for Aux II are commonly conserved in all groups while 3-CyFEs lack the Cys residues corresponding to Aux I cluster. d, Sequence-function space of rSAM/SPASM proteins containing 3-CyFEs (n = 13,151; AS = 75; 40% representative nodes). Nodes are based on maturase type. XncB, DarE, and DynA are annotated.

Figure 11. Summary of xenorceptide biosynthesis, precursor types, phylogeny of maturases, and representative BGCs. a, A phylogenetic tree made by Clustal Omega summarizing gene sequences encoding rSAM/SPASM XyeB proteins associated with a type A XyeA precursor. Sequence logos are shown for XyeA core sequences of each genus. b, Representative xye BGCs from each genus.

Figure 12. Synthetic biology for the production of xenorceptides. a, Production of natural product using strategy 2, engineered His6-A/pET28a(+) + BCDE/pCDFDuet-1 (strategy 2). The precursor constituted of His-tagged XncA leader and YkcA core sequence (His6-$XncA_L$-$YkcA_C$) is co-expressed with XncBCDE. This strategy gave a better yield of the ykc natural product (5) than strategy 1. b, Summary of xenorceptides named xenorceptides A2-A10 (2-10) produced in this study. Characteristic motifs/residues are highlighted in red. Products 9 and 10 could not be isolated due to the low yield.

Figure 13. Biological evaluation of xenorceptide A2 (2). a, Time-kill kinetics of xenorceptide A2 (2) against E. coli M6 over 24 h was determined by agar colony count. Colistin at 2×MIC was tested as a positive control. Black dotted lines indicate the limit of detection (50 CFU/mL). Experiments were repeated on three biologically independent samples. Data are presented as geometric mean ± SE. b, The development of resistance of E. coli M6 against xenorceptide A2 (2) was monitored using serial passage over 14 days. Experiments were repeated three times with different starting bacteria cultures. c, SEM images of E. coli M6 after treatment with xenorceptide A2 (2) at 4× or 8×MIC for 2 h. For each sample slide, at least five independent fields were imaged to ensure representativeness. Magnification = 25,000×. Scale bar = 1 μm. d, Experiment schematics of the mouse peritonitis model infected with E. coli M6 for evaluating the in vivo efficacy of xenorceptide A2 (2). e, Bacteria burden in the peritoneal fluid, blood,

liver, spleen, and kidney of C57BL/6NTac mice (n = 5 mice per treatment group) collected 5 h after treatment with 5 mg/kg xenorceptide A2 (2), 50 mg/kg xenorceptide A2 (2), 5 mg/kg colistin, or saline (vehicle control). Samples were plated onto LB agar and incubated for 18-20 h at 37 °C before colony count. Colony counts of organ tissues were normalized against the average mass of the respective mouse organs. Statistical significance of differences between data groups were evaluated using one-way analysis of variance (ANOVA) followed by Turkey post-hoc test (ns: $p > 0.05$, *: $p \leq 0.05$, **: $p \leq 0.01$).

Figure 14. Synthetic biology for the production of **11** by co-expression of His6-A/pET28a(+) + BCDE/pCDFDuet-1 (strategy 2).

Figure 15. Synthetic biology for the production of **12** by co-expression of His6-A/pET28a(+) + BCDE/pCDFDuet-1 (strategy 2).

Figure 16. Synthetic biology for the production of **13** by co-expression of His6-A/pET28a(+) + BCDE/pCDFDuet-1 (strategy 2).

Figure 17. Summary of Xye Type B and Type D biosynthetic gene clusters and the corresponding sequence of the precursor.

Figure 18. LC-MS analysis of coexpression of His6-XgcA1B and full cluster expression His6-XgcA1B + DEC full-length precursors. (a) XgcA1 sequence with His6-tag. (b) Blue fill shows the truncated leader only existed in full-cluster expression. (c) MS of truncated leader from GG. *A1BDEC=Full-cluster expression, A1B= XgcA1B only.

Figure 19. LC-MS analysis of coexpression of His6-PlcAB digested with trypsin and full cluster expression His6-PlcAB + PlcCDE full-length precursors. (a) PlcA sequence with His6-tag. (b-e) LC-MS analysis of PlcAB and PlcAB + PlcCDE full-length precursors. (b) Blue fill shows the truncated leader only existed in full-cluster expression. (c, d) MS of truncated leader from GG. (e) LC-MS of extracted ion chromatogram (EIC) data of PlcAB and PlcAB + PlcCDE tryptic fragment, the red arrows indicating that the plc precursor in Plc full cluster expression cleavage at GG (red arrow), while PlcAB only expression does not exhibit this cleavage. *ABCDS=Full-cluster expression, AB= PlcAB only

Figure 20. The *xgc* biosynthetic gene cluster, the protein sequence of XgcA1 and XgcA2 are given at right side.

Figure 21. The *phc* biosynthetic gene cluster, the protein sequence of PhcA is given at right side.

Figure 22. (a) The *kcc2* and *kcc1* biosynthetic gene clusters, the protein sequence of Kcc2A and Kcc1A are given at right side. (b) LC-MS analysis of SPE elute fraction of Kcc2AB + Kcc2CDE, with **24-26** indicating Kcc2 products. (c) LC-MS analysis of SPE

elute fraction of Kcc1AB + Kcc2CDE, with **27-29** indicating Kcc1 products.

Figure 23. LC-MS analysis of variants. (a) Co-expression of XgcA2(G-1K) and XgcB, followed by trypsin digestion leads to the formation of compound **22**. (b) Co-expression of Kcc1(G-1E) and Kcc1B, followed by GluC digestion leads to the formation of compound **27** and **28**. (c) Co-expression of Poc_leader/Bbc_core_(G-1K) fusion precursor and PocB, followed by trypsin digestion leads to the formation of compound **30** and **31**. For **31**, b&y ions in MS data suggested the -2D modification is localized to the WSK motif. (d) Co-expression of Poc(G-1R) and PocB, followed by trypsin digestion leads to the formation of compound **32** and **33**. For **33**, b&y ions in MS data suggested the -2D modification is localized to the WSR motif.

Figure 24. Structure of compound **24**. Peptide sequences for compound **24** (top), and structure of residues +5 to +12 of fragment (bottom). Blue connectors in the core peptide sequences indicate modifications (−2 Da) detected and localized by LC-MS/MS.

Figure 25. Key features of Kcc2-4D HMBC (a) and COSY (b), showing the correlation between Trp5-C6 and Arg7β and Trp10-C6 and Lys12β C-C bond formation.

Figure 26. Structure elucidation of xenorceptide A2 (**2**). a, Key 2D NMR correlation of 2. b, Conformational analysis and NOE correlations for WVN (left), FAR (center), and WSK (right) motifs.

Figure 27. Structure elucidation of xenorceptide A3 (**3**). a, Key 2D NMR correlation of 3. b, Conformational analysis and NOE correlations for WVN (left), FAN (center), and WTK (right) motifs.

Figure 28. Structure elucidation of xenorceptide A4 (**4**). a, Key 2D NMR correlation of 4. b, Conformational analysis and NOE correlations for WVN (left), YAR (center), and WTK (right) motifs.

Figure 29. 1H NMR spectrum of xenorceptide A2. Acquired at 800 MHz in DMSO-d6 at 298 K.

Figure 30. TOCSY xenorceptide A2. Acquired at 800 MHz in DMSO-d6 at 298 K.

Figure 31. Phase-sensitive NOESY spectrum of xenorceptide A2. Acquired at 800 MHz in DMSO-d6 at 298 K.

Figure 32. HSQC spectrum of xenorceptide A2. Acquired at 800 MHz in DMSO-d6 at 298 K.

Figure 33. HMBC spectrum of xenorceptide A2. Acquired at 800 MHz in DMSO-d6 at 298 K.

Figure 34. 1H NMR spectrum of xenorceptide A3. Acquired at 400 MHz in DMSO-d6+0.3% TFA-d at 298 K.

Figure 35. COSY spectrum of xenorceptide A3. Acquired at 400 MHz in DMSO-d6 + 0.3% TFA-d at 298 K.

Figure 36. TOCSY spectrum of xenorceptide A3. Acquired at 400 MHz in DMSO-d6 + 0.3% TFA-d at 298 K.

Figure 37. Phase-sensitive NOESY spectrum of xenorceptide A3. Acquired at 400 MHz in DMSO-d6 + 0.3% TFA-d at 298 K.

Figure 38. Edited-HSQC spectrum of xenorceptide A3. Acquired at 400 MHz in DMSO-d6 + 0.3% TFA-d at 298 K.

Figure 39. HMBC spectrum of xenorceptide A3. Acquired at 400 MHz in DMSO-d6 + 0.3% TFA-d at 298 K.

Figure 40. 1H NMR spectrum of xenorceptide A4. Acquired at 400 MHz in DMSO-d6 + 0.2% TFA-d at 298 K.

Figure 41. COSY spectrum of xenorceptide A4. Acquired at 400 MHz in DMSO-d6 + 0.2% TFA-d at 298 K.

Figure 42. TOCSY spectrum of xenorceptide A4. Acquired at 400 MHz in DMSO-d6 + 0.2% TFA-d at 298 K.

Figure 43. Phase-sensitive NOESY spectrum of xenorceptide A4. Acquired at 400 MHz in DMSO-d6 + 0.2% TFA-d at 298 K.

Figure 44. Edited-HSQC spectrum of xenorceptide A4. Acquired at 400 MHz in DMSO-d6 + 0.2% TFA-d at 298 K.

Figure 45. HMBC spectrum of xenorceptide A4. Acquired at 400 MHz in DMSO-d6 + 0.2% TFA-d at 298 K.

Figure 46. 1H spectrum of product xenorceptide D1. Acquired at 400 MHz in DMSO at 298 K.

Figure 47. COSY spectrum of product xenorceptide D1. Acquired at 400 MHz in DMSO at 298 K.

Figure 48. TOSCY spectrum of product xenorceptide D1. Acquired at 400 MHz in DMSO at 298 K.

Figure 49. HSQC spectrum of product xenorceptide D1. Acquired at 400 MHz in DMSO at 298 K.

Figure 50. HMBC spectrum of product xenorceptide D1. Acquired at 400 MHz in DMSO at 298 K.

Figure 51. TOSCY spectrum of product xenorceptide D1. Acquired at 400 MHz in DMSO at 298 K.

Detailed description

The term "cyclophane group" or "cyclophane" may be used interchangeably to refer to a macrocycle or ring consisting of an aromatic unit (aryl or heteroaryl) and an optionally substituted aliphatic chain that forms a bridge between two non-adjacent positions of the aromatic ring. For example, the "cyclophane group" or "cyclophane" can refer to a macrocycle or ring formed when an aromatic unit in an aromatic amino acid X_1 (such as W, F, Y or H) in a peptide comprising a 3 residue motif X_1-X_2-X_3 is joined to a Cβ in X_3 via a carbon to carbon bond.

The terms "polypeptide", "peptides" and "protein" are used interchangeably and include any polymer of amino acids (dipeptide or greater) linked through peptide bonds or modified peptide bonds, whether produced naturally or synthetically. The polypeptides of the invention may comprise non-peptidic components, such as carbohydrate or fatty acid groups.

The term "amino acid" refers to naturally occurring and non-natural amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally encoded amino acids are the 20 common amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine) and pyrrolysine and selenocysteine. Amino acid analogs refer to compounds that have the same basic chemical structure as a naturally occurring amino acid, by way of example, an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group. Such analogs may have modified R groups (by way of example, norleucine) or may have modified peptide backbones, while still retaining the same basic chemical structure as a naturally occurring amino acid. Non-limiting examples of amino acid analogs include homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. The amino acid as referred to herein may be a D or L amino acid. The amino acid may also be a β-amino acid. The term "amino acid" can include D-amino acids, α,α-disubstituted amino acids, N-alkyl amino acids, homo-amino acids, dehydroamino acids, aromatic amino acids (other than phenylalanine, tyrosine and tryptophan), and ortho-, meta- or para-aminobenzoic acid, non-conventional amino acids such as compounds which have an amine and carboxyl functional group separated in a 1,3 or larger substitution pattern,

such as β-alanine, γ-amino butyric acid, Freidinger lactam, the bicyclic dipeptide (BTD) , amino- methyl benzoic acid and others well known in the art. Statine-like isosteres, hydroxyethylene isosteres, reduced amide bond isosteres, thioamide isosteres, urea isosteres, carbamate isosteres, thioether isosteres, vinyl isosteres and other amide bond isosteres known to the art are also included.

A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, which can be generally sub-classified as follows:

Table 1: Amino Acid Subclassification

Sub-classes	*Amino acids*
Acidic	Aspartic acid, Glutamic acid
Basic	Noncyclic: Arginine, Lysine; Cyclic: Histidine
Charged	Aspartic acid, Glutamic acid, Arginine, Lysine, Histidine
Small	Glycine, Serine, Alanine, Threonine, Proline
Polar/neutral	Asparagine, Histidine, Glutamine, Cysteine, Serine, Threonine
Polar/large	Asparagine, Glutamine
Hydrophobic	Tyrosine, Valine, Isoleucine, Leucine, Methionine, Phenylalanine, Tryptophan
Aromatic	Tryptophan, Tyrosine, Phenylalanine, Histidine
Residues that influence chain orientation	Glycine and Proline

Conservative amino acid substitution also includes groupings based on side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. For example, it is reasonable to

expect that replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the properties of the resulting variant polypeptide. Whether an amino acid change results in a functional polypeptide can readily be determined by assaying its activity. Conservative substitutions are shown in Table 2 under the heading of exemplary and preferred substitutions. Amino acid substitutions falling within the scope of the invention, are, in general, accomplished by selecting substitutions that do not differ significantly in their effect on maintaining (a) the structure of the peptide backbone in the area of the substitution, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. After the substitutions are introduced, the variants are screened for biological activity.

Table 2: Exemplary and Preferred Amino Acid Substitutions

Original Residue	*Exemplary Substitutions*	*Preferred Substitutions*
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln, His, Lys, Arg	Gln
Asp	Glu	Glu
Cys	Ser	Ser
Gln	Asn, His, Lys,	Asn
Glu	Asp, Lys	Asp
Gly	Pro	Pro
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleu	Leu
Leu	Norleu, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, Gln, Asn	Arg
Met	Leu, Ile, Phe	Leu
Phe	Leu, Val, Ile, Ala	Leu
Pro	Gly	Gly
Ser	Thr	Thr
Thr	Ser	Ser
Trp	Tyr	Tyr

Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Leu, Met, Phe, Ala, Norleu	Leu

Unnatural amino acids may include amino acids which are not in the L conformation. These can include non-α amino acids such as β amino acids and D amino acids. Unnatural amino acids incorporated into peptides may include 1) a ketone reactive group (as found in para or meta acetyl-phenylalanine) that can be specifically reacted with hydrazines, hydroxylamines and their derivatives (Addition of the keto reactive group to the genetic code of Escherichia coli. Wang L, Zhang Z, Brock A, Schultz P G. Proc Natl Acad Sci USA. 2003 Jan. 7; 100(1):56-61; Bioorg Med Chem Lett. 2006 Oct. 15; 16(20):5356-9. Genetic introduction of a diketone-containing amino acid into proteins. Zeng H, Xie J, Schultz P G), 2) azides (as found in p-azido-phenylalanine) that can be reacted with alkynes via copper catalysed "click chemistry" or strain promoted (3+2) cyloadditions to form the corresponding triazoles (Addition of p-azido-L-phenylalanine to the genetic code of Escherichia coli. Chin J W, Santoro S W, Martin A B, King D S, Wang L, Schultz P G. J Am Chem Soc. 2002 Aug. 7; 124(31):9026-7; Adding amino acids with novel reactivity to the genetic code of Saccharomyces cerevisiae. Deiters A, Cropp T A, Mukherji M, Chin J W, Anderson J C, Schultz P G. J Am Chem Soc. 2003 Oct. 1; 125(39):11782-3), or azides that can be reacted with aryl phosphines, via a Staudinger ligation (Selective Staudinger modification of proteins containing p-azidophenylalanine. Tsao M L, Tian F, Schultz P G. Chembiochem. 2005 December; 6(12):2147-9), to form the corresponding amides, 4) Alkynes that can be reacted with azides to form the corresponding triazole (In vivo incorporation of an alkyne into proteins in Escherichia coli. Deiters A, Schultz P G. Bioorg Med Chem Lett. 2005 Mar. 1; 15(5):1521-4), 5) Boronic acids (boronates) than can be specifically reacted with compounds containing more than one appropriately spaced hydroxyl group or undergo palladium mediated coupling with halogenated compounds (Angew Chem Int Ed Engl. 2008; 47(43):8220-3. A genetically encoded boronate-containing amino acid, Brustad E, Bushey M L, Lee J W, Groff D, Liu W, Schultz P G), 6) Metal chelating amino acids, including those bearing bipyridyls, that can specifically co-ordinate a metal ion (Angew Chem Int Ed Engl. 2007; 46(48):9239-42. A genetically encoded bidentate, metal-binding amino acid. Xie J, Liu W, Schultz P G).

The majority of strains on the WHOs Priority Pathogens List for R&D of new antibiotics belong to the family Enterobactericiae and include Klebsiella pneumoniae, Escherichia

coli, Enterobacter spp., Serratia spp., Proteus spp., Providencia spp., and Morganella spp. These strains are multi-drug resistant and lead to severe and deadly infections in hospitals and nursing homes. The discovery of new antibiotics with the ability to treat these infections will have significant impact in the clinic and can save thousands of lives annually.

The present invention is predicated on the understanding that RiPP cyclophane-containing natural products may be a source of antibiotics against Gram-negative pathogens. For example, Darobactin was isolated from Photorhabdus khanii in efforts targeting animal associated symbionts as a promising source of new antibiotics. The structure of darobactin is composed of two fused three-residue cyclophanes and an ether linkage (Figure 10a). Homologues of the maturase DarE, have also been characterized to install an ether which is a characteristic feature for this class of maturases and products (Figure 10b). Dynobactin was recently reported by a research group by expanding on this class of natural products bioinformatically and optimizing the purification protocol by testing of purified fractions. Dynobactin contains one four-residue and one three-residue cyclophane with the latter incorporating an imidazole via Nε2 linkage (Figure 10a). Sequence comparison of DynA precursors shows the 4-residue cyclophane is likely conserved while the second cyclophane appears to be formed between two aromatic residues (Figure 10b).

In an alternative approach to natural products drug discovery, the inventors pursued identification of a new RiPP family prior to knowledge of the bioactivity of the natural products. The rationale was that new RiPP families will contain new products for screening platforms and biosynthetic enzymes that could be applied for making drug-like molecules. To do this the inventors systematically characterized three unique TIGRFAMs annotated as rSAM/SPASM maturases (Xye, TIGR04996: Grr, TIGR04261; and Fxs, TIGR04269) and found they are unified in their ability to catalyze 3-residue cyclophane formation. Cyclophane formation occurs via a C(sp^2)-Cβ(sp^3) bond between an aromatic ring and β-position on 3-residue Ω1-X2-X3 motifs where all aromatic residues (Phe, Trp, Tyr, and His) appear at the Ω1 position (Figure 10b). Collectively, the maturases is referred to as 3-residue cyclophane forming enzymes (3-CyFEs). 3-CyFEs can be differentiated from DarE, DynA, and other radical SAM/SPASM maturases by the lack of Cys residues that bind auxiliary cluster 1 of the SPASM domain (Figure 10c). BGCs that contain at least one 3-CyFE define a new family of RiPPs are termed as

triceptides. 3-CyFEs were localized within a region of rSAM/SPASM sequence-function space and analysis of this biosynthetic landscape allowed the identification of ~4000 triceptide precursors which are broadly distributed in bacteria (Figure 10d). With a new RiPP family identified the inventors focused on a specific maturase system for antibiotic discovery.

As the activity and function for triceptides was unknown, the Xye maturase systems (GenProp1090) as a source of potential antibiotics for several reasons. First, *xye* BGCs are reminiscent of Class I bacteriocins, a well-known source of antibacterial peptides. Shared biosynthetic features include precursors encoding a Gly-Gly motif that separates the leader and core peptide, and protease/transporter proteins that cleave and export the mature RiPP (Figure 10a and 1a). Second, most xye BGC-containing bacteria are isolated from human or animal microbiomes. Since these end products are likely secreted and act in a biological environment similar to that experienced by clinically used antibiotics, the inventors hypothesize that these molecules would have evolved ideal drug-like features. Third, the inventors previously demonstrated production of xenorceptide A1, as a representative from the Xye maturase system. To their knowledge, xenorceptide A1 is the first characterized triceptide natural product. The inventors collectively refer to the triceptides derived from the Xye maturase systems as xenorceptides. Although xenorceptide A1 was not active when tested against several bacterial strains, the inventors believed that the production of xenorceptide A1 provided an entry point to produce and study this subfamily further. The inventors hypothesized that the diversity in bacterial and core sequences within XyeA precursors had the potential to generate peptide antibiotics.

The bioinformatic analysis and synthetic biology enabled production of xenorceptides is now disclosed herein. Screening of the natural products against Gram-negative and Gram-positive pathogens revealed xenorceptide A2 which was subjected to further biological evaluation. This study adds Xenorceptides to the RiPP cyclophane antibiotic class, and identified xenorceptide A2 as an antibiotics candidate.

The present invention provides a polypeptide comprising:

a) a first three residue motif (from a N-terminus) and a second three residue motif, the first and second three residue motif optionally separated by 1 to 3 amino acid residue; and

b) at least two C-terminus residues;
wherein the three residue motif is each represented by X_1-X_2-X_3;
wherein each X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine, an unnatural aromatic amino acid residue or a derivative thereof;
wherein each X_2 and X_3 are independently any amino acid residue;
wherein X_1 and X_3 in each motif are connected to form a cyclophane moiety;
wherein at least one of the two C-terminus residues is an aromatic residue.

The present invention provides a polypeptide comprising:
a) a first three residue motif (from a N-terminus) and a second three residue motif, the first and second three residue motif optionally separated by 1 to 3 amino acid residue; and
b) at least two C-terminus residues;
wherein the three residue motif is each represented by X_1-X_2-X_3;
wherein each X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine, or an unnatural aromatic amino acid residue;
wherein each X_2 and X_3 are independently any amino acid residue;
wherein X_1 and X_3 in each motif are connected to form a cyclophane moiety;
wherein at least one of the two C-terminus residues is an aromatic residue; and
wherein X_1 and X_3 in the second motif are connected via phenylene to form a cyclophane moiety.

A cyclophane is a hydrocarbon consisting of an aromatic unit and a chain that forms a bridge between two non-adjacent positions of the aromatic ring.

When the polypeptide comprises two three residue motifs, the two three residue motifs may be referred to as a first three residue motif (from the N-terminus) and a second three residue motif (following the first motif).

The three residue motif may be each represented by X_1-X_2-X_3.

The polypeptide is modified such that X_1 and X_3 in each motif are linked. The linkage may be via W, F, Y or H to form imidazolylene, indolylene or phenylene-bridged cyclophanes. The modified polypeptide may, for example, display restricted rotation of the aromatic ring and induce planar chirality in the asymmetric indole bridge. In some

embodiments, X_1 and X_3 are connected via phenylene or indolylene to form a cyclophane moiety. In some embodiments, X_1 and X_3 in the second motif are connected via phenylene to form a cyclophane moiety.

In some embodiments, X_1 is each X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine, an unnatural aromatic amino acid residue or a derivative thereof. In some embodiments, the first X_1 is a residue selected from tryptophan, phenylalanine, tyrosine, histidine, an unnatural aromatic amino acid residue or a derivative thereof. In some embodiments, the first X_1 is a residue selected from tryptophan, phenylalanine, tyrosine, histidine or a derivative thereof. In some embodiments, the first X_1 is a residue selected from tryptophan, phenylalanine or a derivative thereof. In some embodiments, the second X_1 is a residue selected from tryptophan, phenylalanine, tyrosine, histidine, an unnatural aromatic amino acid residue or a derivative thereof. In some embodiments, the second X_1 is a residue selected from tryptophan, phenylalanine, tyrosine, histidine or a derivative thereof. In some embodiments, the second X_1 is a residue selected from tryptophan, phenylalanine, tyrosine or a derivative thereof. In some embodiments, the second X_1 is a residue selected from phenylalanine, tyrosine or a derivative thereof.

X_2 and X_3 may each independently be any amino acid. In some embodiments, X_2 is I, G, E, Y, V, L, A, D, S, T, N or Q. X_3 may be a non-aromatic amino acid. In some embodiments, X_3 is an amino acid that is not W, F, Y or H. In some embodiments, X_3 is N, R, S, D, Q or K. In somne embodiment, X_3 is N, R or K.

In some embodiments, X_2 is I, G, E, Y, V, L, A, D, S, T, N or Q, and X_3 is N, R, S, D or K. In some embodiments, X_2 is I, G, E, Y, V, L, A, D, S, T, N or Q, and X_3 is N, R or K.

In some embodiments, the first and second three residue motifs are separated by 0 amino acid residue. In some embodiments, the first and second three residue motifs are separated by 1 to 3 amino acid residue. In some embodiments, the two three residue motifs are separated by 1 to 2 amino acid residue. In some embodiments, the two three residue motifs is separated by 1, 2 or 3 amino acid residue.

The first and second three residue motifs may be separated by any type of amino acid residue, natural or non-natural. In some embodiments, the two three residue motifs is

separated by a residue selected from A, V, Y, F, T, Q, G, L, D, or S. In some embodiments, the two three residue motifs is separated by A.

In some embodiments, the first three residue motif is not fused with the second three residue motif other than via 1-3 amino acid residues or an amide bond. In other embodiments, the cyclophane moiety in the first three residue motif is not fused to the cyclophane moiety in the second three residue motif. In some embodiments, the cyclophane moieties connecting X_1 and X_3 in each motif are not fused to each other. In this regard, in contrast to darobactin for example, the polypeptide of the present invention does not comprise linked three-residue cyclophanes. The polypeptide of the present invention also does not comprise an ether linkage between the three-residue cyclophanes motifs.

The C-terminus comprises at least two residues. These residues do not form part of the three residue motif. In some embodiments, the C-terminus comprises at least three residues, or at least four residues. In other embodiments, the C-terminus comprises 2 to 8 residues, 2 to 7 residues, 2 to 6 residues, 2 to 5 residues, or 2 to 4 residues. In some embodiments, the C-terminus comprises at least three residues.

At least one of the two C-terminus residues is an aromatic residue. For example, at least one of the C-terminus residue may be tryptophan, tyrosine, phenylalanine, or histidine. In some embodiments, at least one of the two C-terminus residues is a polar and/or basic residue. In some embodiments, the C-terminus comprises an aromatic residue and a polar and/or basic residue.

It was found that having at least an aromatic residue at the C-terminus improves the anti-bacterial property of the polypeptide.

In some embodiments, the polypeptide comprises at least three three residue motifs. In this regard, the three three residue motifs may be referred to as a first motif (from the N-terminus), a second motif (following the first motif), and a third motif (following the second motif and in proximity to the C-terminus).

In some embodiments, the third X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine, an unnatural aromatic amino acid residue or a

derivative thereof. In some embodiments, the third X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine or a derivative thereof. In some embodiments, the third X_1 is a residue independently selected from tryptophan, phenylalanine or a derivative thereof.

In some embodiments, when the polypeptide comprises a third three residue motifs, X_3 of the second motif (from the N-terminus) and X_1 of the third motif are covalently bonded to each other via an amide bond. Accordingly, the second motif and the third motif are not separated by any residue.

In one embodiment, the polypeptide is a linear polypeptide. The polypeptide may be of any sequence length, having any number of residues at the N-terminus or C-terminus as long as it comprises at least two three residue motif optionally separated by 1 to 3 amino acid residue and at least two C-terminus residues.

In some embodiments, the polypeptide is represented by Formula (I):

$$X_1\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \qquad (I)$$

wherein each X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine or an unnatural aromatic amino acid residue;
wherein each X_2 and X_3 are independently any amino acid residue;
wherein X_n is an amide bond or 1 to 3 amino acid residue; and
wherein X_m is at least two C-terminus residues.

In some embodiments, the polypeptide is represented by Formula (I'):

$$X_1\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_{m1}\text{-}X_{m2} \qquad (I')$$

wherein X_{m1} is a first C-terminus residue; and
X_{m2} is a second C-terminus residue.

In some embodiments, each X_2 is an amino acid residue, the amino acid independently selected from leucine, isoleucine, valine, alanine, proline, serine, lysine, asparagine, phenylalanine, aspartic acid or a derivative thereof.

In some embodiments, each X_3 is an amino acid residue, the amino acid independently selected from lysine, glutamine, asparagine, arginine or a derivative thereof. In some embodiments, each X_3 is an amino acid residue, the amino acid independently selected

from lysine, asparagine, arginine or a derivative thereof.

In some embodiments, the polypeptide is represented by Formula (II):

$$X_1\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad (II)$$

wherein each X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine or an unnatural aromatic amino acid residue;
wherein each X_2 and X_3 are independently any amino acid residue;
wherein X_n is an amide bond or 1 to 3 amino acid residue; and
wherein X_m is at least two C-terminus residues.

In some embodiments, the polypeptide is represented by Formula (II'):

$$X_1\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_{m1}\text{-}X_{m2} \quad (II')$$

wherein X_{m1} is a first C-terminus residue; and
X_{m2} is a second C-terminus residue.

In some embodiments, each X_2 is an amino acid residue, the amino acid independently selected from valine, isoleucine, phenylalanine, tryptophan, alanine, leucine, glycine, serine, proline, threonine, aspartic acid, asparagine, glutamic acid, arginine or a derivative thereof.

In some embodiments, each X_3 is an amino acid residue, the amino acid independently selected from arginine, lysine, asparagine or a derivative thereof.

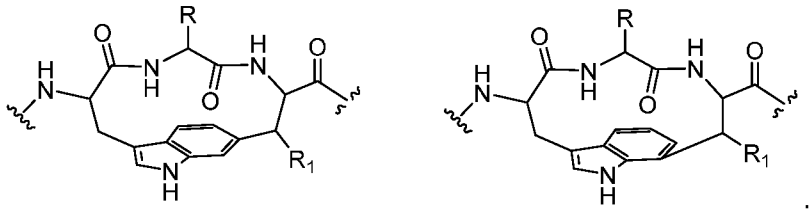
In some embodiments, X_1 and X_3 in the first motif are connected via indolylene to form a cyclophane moiety. In some embodiments, X_1 and X_3 in the second motif are connected via phenylene to form a cyclophane moiety.

In some embodiments, the polypeptide is represented by Formula (Ia) or (IIa):

$$W\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad (Ia)$$

$$W\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad (IIa).$$

In some embodiments, X_1 is W. In some embodiments, X_1 of the first motif is W. In some embodiments, when X_1 is W, X_1 (or W) is connected to X_3 via a 3,6 or 3,7 disubstituted indolylene moiety. This may for example be represented pictorially as follows:



In some embodiments, the polyp eptide is represented by Formula (Ia') or (IIa'):

$W\text{-}X_2\text{-}N/K/R\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m$ (Ia)

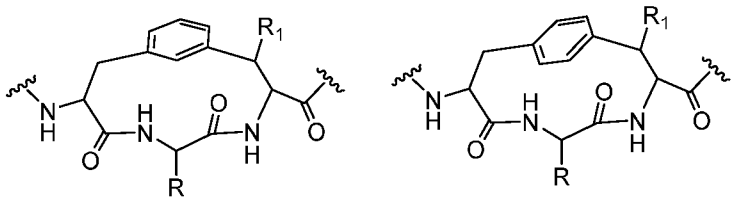
$W\text{-}X_2\text{-}N/K/R\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m$ (IIa).

In some embodiments, the polypeptide is represented by Formula (Ib) or (IIb):

$W\text{-}X_2\text{-}X_3\text{-}X_n\text{-}F/Y\text{-}X_2\text{-}X_3\text{-}X_m$ (Ib)

$W\text{-}X_2\text{-}X_3\text{-}X_n\text{-}F/Y\text{-}X_2\text{-}X_3\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m$ (IIb).

In some embodiments, X_1 is F or Y. In some embodiments, X_1 of the second motif is F or Y. In some embodiments, when X_1 is F or Y, X_1 (being F or Y) is connected to X_3 via a 1,3 or 1,4 disubstituted phenylene moiety. The 1,4 disubstituted phenylene moiety may for example be represented pictorially as follows:



In some embodiments, the polypeptide is represented by Formula (Ib') or (IIb'):

$W\text{-}X_2\text{-}N/K/R\text{-}X_n\text{-}F/Y\text{-}X_2\text{-}N/K/R\text{-}X_m$ (Ib')

$W\text{-}X_2\text{-}N/K/R\text{-}X_n\text{-}F/Y\text{-}X_2\text{-}N/K/R\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m$ (IIb').

In some embodiments, the polypeptide is represented by Formula (IIc):

$W\text{-}X_2\text{-}X_3\text{-}X_n\text{-}F/Y\text{-}X_2\text{-}X_3\text{-}W\text{-}X_2\text{-}X_3\text{-}X_m$ (IIc).

In some embodiments, the polypeptide is represented by Formula (IIc):

$W\text{-}X_2\text{-}N/K/R\text{-}X_n\text{-}F/Y\text{-}X_2\text{-}N/K/R\text{-}W\text{-}X_2\text{-}N/K/R\text{-}X_m$ (IIc').

In some embodiments, when X_1 in the first motif is F, the polypeptide is represented by Formula (Id) or (IId):

$$F\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad (Id)$$

$$F\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad (IId).$$

Such polypeptides may be Type D peptides.

In some embodiments, the polypeptide is represented by Formula (Id') or (IId'):

$$F\text{-}X_2\text{-}N/K\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad (Id)$$

$$F\text{-}X_2\text{-}N/K\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad (IId).$$

In some embodiments, the polypeptide is represented by Formula (Ie) or (IIe):

$$F\text{-}X_2\text{-}X_3\text{-}X_n\text{-}W\text{-}X_2\text{-}X_3\text{-}X_m \quad (Ie)$$

$$F\text{-}X_2\text{-}X_3\text{-}X_n\text{-}W\text{-}X_2\text{-}X_3\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad (IIe).$$

In some embodiments, the polypeptide comprises 3 three residue motifs, wherein X_1 of the second three residue motif is F, X_3 of the second and third three residue motifs are independently basic amino acid residues, and at least one of the two C-terminus residues is an aromatic residue.

In some embodiments, the polypeptide is selected from Table 3:

Table 3: Xenorceptides

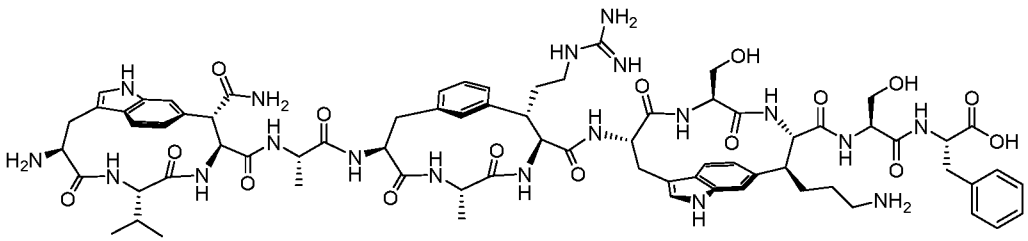
SEQ ID	Type[e]	xenorceptide[f]	Bacterial strain	Core Sequence[a]	Length[d]	MIC (E. coli)[b]
1	A		Xenorhabdus sp. NBAII XenSa04	**W**VNA**F**AN**W**SKAL	51	
2	A		Xenorhabdus stockiae DSM 17904	**W**VNA**F**GN**W**SKSL	51	
3	A	A6 (**6**)	Xenorhabdus sp. BG5	**W**VNA**F**AN**W**SKSF	51	
4	A		Kosakonia cowanii pasteuri	**W**VNA**F**AR**W**GKSF	51	
5	A		Yersinia sp. Marseille-Q3913	**W**VNA**F**AR**W**GRAF	51	
6	A	A5 (**5**)	Yersinia kristensenii IP6945	**W**VNA**F**VN**W**PKSF	51	

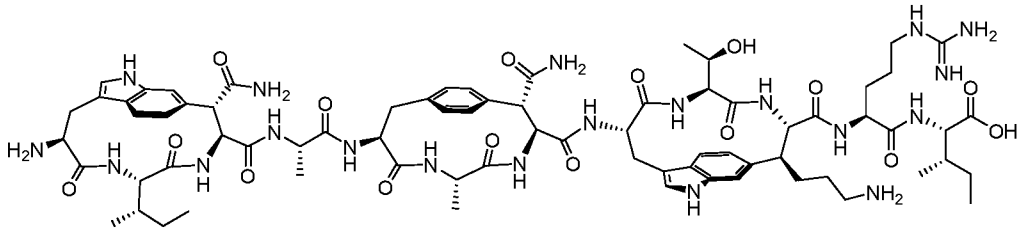
7	A		Yersinia bercovieri 127/84	**W**INA**F**AR**W**GRAF	51	
8	A	A2 (**2**)	Serratia marcescens CAV1761	**W**VNA**F**AR**W**SKSF	51	
9	A		Yersinia enterocolitica PS23	**W**VNA**F**VN**W**TKSF	51	
10	A		Xenorhabdus bovienii CS03	**W**VNV**F**AR**W**DKAI	51	
11	A		Erwinia	**W**VNA**F**AN**W**TKRI	51	
12	A		Yersinia aleksiciae	**W**VNA**F**LR**W**GKSF	51	
13	A	A3 (**3**)	Erwinia toletana DAPP-PG 735	**W**INA**F**AN**W**TKRI	51	8
14	A		Photorhabdus heterorhabditis ETL	**W**VNA**F**AK**W**TKRI	51	
15	A		Salmonella enterica	**W**VNF**F**AK**F**TKSF	52	
16	A		Yersinia aldovae IP23238	**W**VNA**F**LN**W**SRSF	51	
17	A		Erwinia sp. E602	**W**VNA**F**AN**W**PKRF	53	
18	A		Yersinia frederiksenii RS-42	**W**VNA**F**LN**W**PRSF	51	
19	A	A8 (**8**)	Aeromonas jandaei CN17A0119	**W**VNA**F**AN**W**TKRF	51	
20	A	A10 (**10**)	Vibrio sagamiensis NBRC 104589	**W**VNA**F**AR**F**TKRF	55	
21	A		Xenorhabdus japonica DSM 16522	**W**INV**F**AR**W**NRAI	51	
22	A	A9 (**9**)	Providencia huaxiensis Pvs2	**W**VNV**F**AR**W**DKQI	51	
23	A	A7 (**7**)	Sodalis sp. dw_96	**W**VNA**F**AR**W**DKKF	51	
24	A		Xenorhabdus bovienii str. oregonense	**W**LNV**F**VR**W**DRAI	51	
25	A	A4 (**4**)	Photorhabdus australis DSM 17609	**W**VNA**Y**AR**W**TNRF	56	
26	A		Photorhabdus heterorhabditis SF41	**W**VNA**Y**AR**W**TKRF	51	8
27	A		Yersinia mollaretii SCPM-O-B-7610	AG**W**INA**F**GN**W**TKSF	53	
28	A		Yersinia mollaretii	AG**W**INA**F**AN**W**TKSF	53	
29	A		Yersinia kristensenii	AG**W**IKA**F**GN**W**SRSF	53	
30	A	A11 (**11**)	Serratia marcescens 90-166	**W**VNA**F**AR**W**SRRW	51	1
31	A		Yersinia mollaretii SCPM-O-B-7598	AG**W**INA**F**AN**W**TRSF	53	
32	A	A1 (**1**)	Xenorhabdus nematophila SC 0516	**W**INA**F**GN**W**ERAFH	52	64
33	A		Yersinia enterocolitica E701	AG**W**IKV**F**GN**W**SRSF	50	
34	A		Serratia marcescens ID149856	**W**VNV**F**AR**W**SRRW	51	
35	A		Serratia sp. DD3	AG**W**IRA**F**AN**W**SRSF	53	4[c]
36	A		Mixta theicola QC88-366	G**W**FRA**Y**LR**W**SRSF	54	

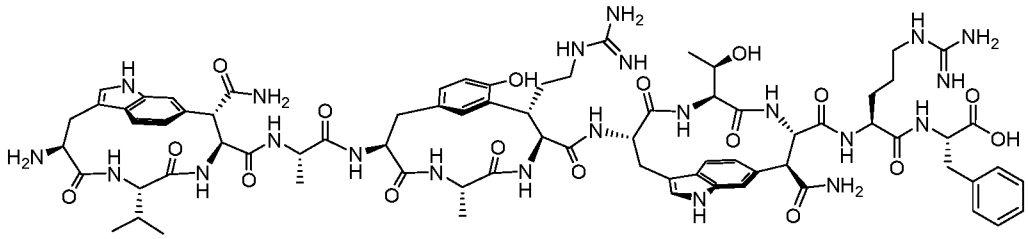
37	A		Gilliamella sp. Lep-s5	**W**WRA**Y**AR**W**RRSF	54	
38	A	A12-1 (**12**)	Engineered sequence of A-34	**W**VNA**F**AR**W**SKRW	52	2
39	A	A12-2 (**13**)	Engineered sequence of A-34	**W**VNA**F**AR**W**SKRF	52	1
40	B	B1	Photorhabdus laumondii	GDR**W**LK**W**IKNH	48	
41	B		Kosakonia cowanii pasteuri	DGR**W**LQ**W**IKNH	48	
42	C		Yersinia	**W**VNA**F**LN	46	
43	D		Bordetella genomosp. 11 AU8856	VGG**F**ANAS**W**PKSF	53	
44	D		Bordetella bronchialis AU17976	VGG**F**ANAT**W**SKSF	53	
45	D		Bordetella genomosp. 9 AU14267	VGG**F**ANAT**W**PKSF	53	
46	D		Providencia rettgeri 2020EL-00052	KSEAAGG**W**VNFQ**W**KNSW	50	
47	D		Pandoraea oxalativorans	NV**F**VNATWSRAM	52	
48	D		Erythrobacter	**W**SRTV**F**NRVRPV	45	
49	D		Sodalis sp. dw_96	AGNDG**W**VKFG**W**KKKF	45	
50	D	D1	Kosakonia cowanii pasteuri	RGEG**W**VRAY**W**AKRF	49	
51	D		Bartonella	RGQGYVRFIFRRSF	50	
52	D		Photorhabdus heterorhabditis	KPGEG**W**VNFT**W**NKSF	48	
53	D		Erwinia	**W**VNA**F**ANRTMGFLFKL	55	
54	D		Xenorhabdus griffiniae VH1	ASTAET**W**FKLD**W**KKSF	49	
55	D	D2	Xenorhabdus griffiniae VH1	SSDDDGI**F**FKTT**W**DRR	49	
56	D		Burkholderia	ADSQPKARAWFANASFSKRF	56	
57	D		Trinickia	VESQSKPRAWFANSSFSKRF	56	
58	D		Burkholderia	ASSQANSRGWFANATWSKAWR	57	
59	D		Pandoraea norimbergensis	NA**F**VNAT**W**SRAM		
60	D		Pandoraea terrigena LMG 31013	NV**F**VNAT**W**SRAI		

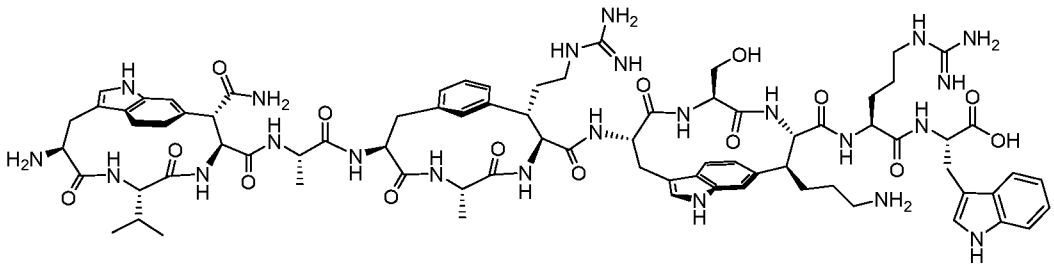
[a]Bold residues indicate aromatic amino acids predicted to be in cyclophane
[b]MIC (μg/mL) indicates the product has been produced and tested against *E. coli*.
[c]Represents the Serraceptide product, aka Serraceptide.
[d]length of a representative precursor encoding each core peptide
[e] Precursor Type and Series of xenorceptide A-D
[f] Xenorceptide compound numbers and abbreviated numbers used in figures (in brackets)

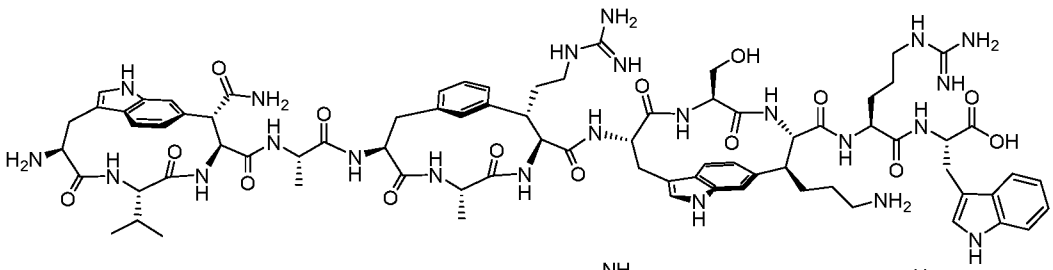
In some embodiments, the polypeptide is selected from:

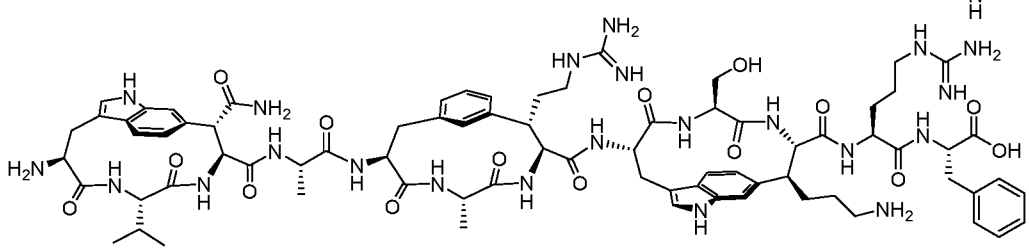


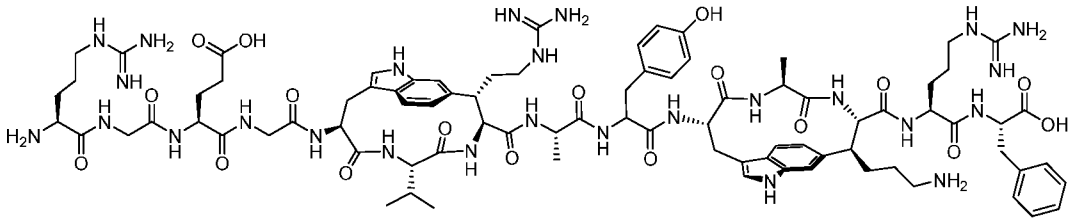












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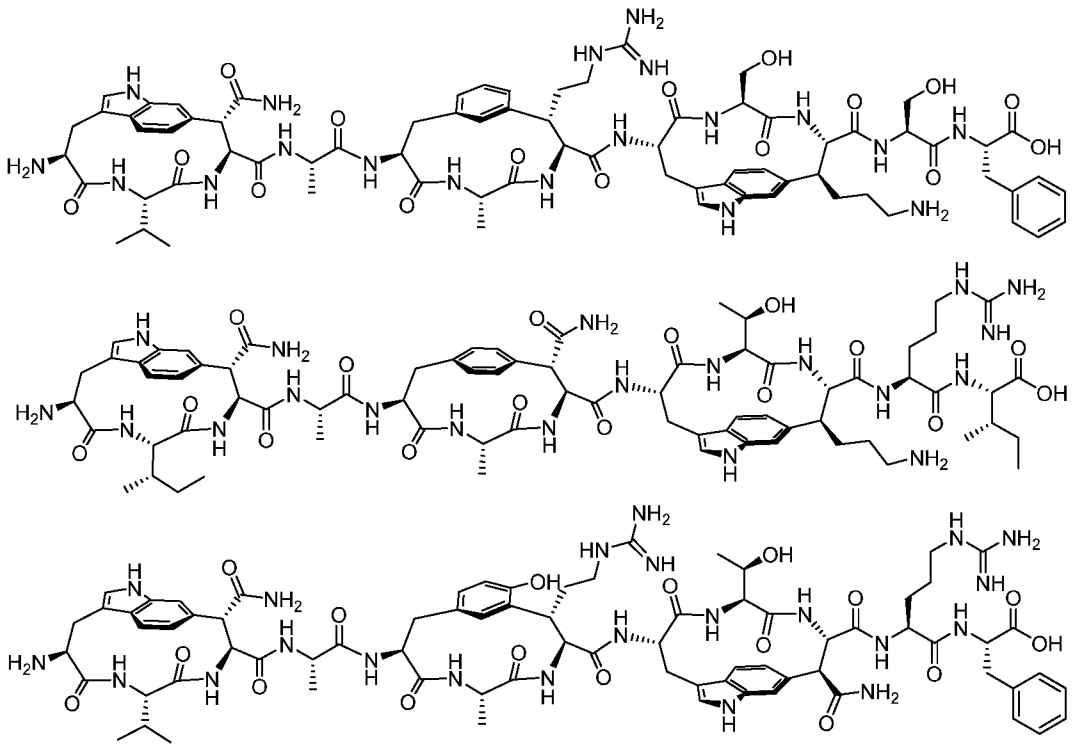
In some embodiments, the polypeptide is selected from **W**VNA**F**AR**W**SKSF (2, SEQ ID 8), **W**INA**F**AN**W**TKRI (3, SEQ ID 13) and **W**VNA**Y**AR**W**TKRF (4, SEQ ID 25). The cyclophane is formed between W and N, F and R, F and N, Y and R, and W and K. In some embodiments, the polypeptide is selected from:



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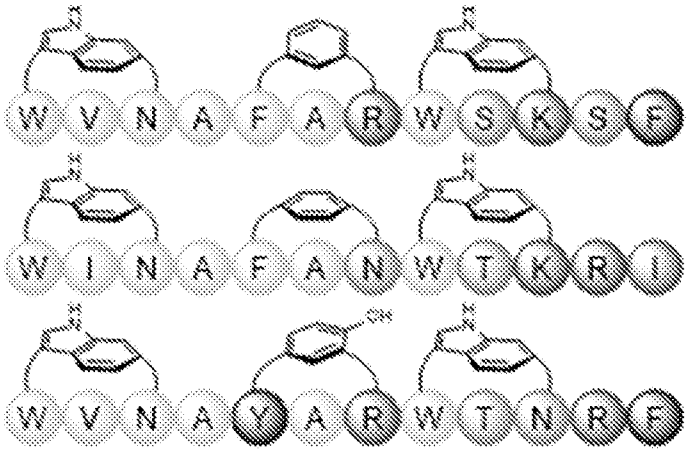
For simplicity, the above three polypeptide can be represented pictorially as follows:



In some embodiments, the polypeptide is characterised by an antibacterial activity. In some embodiments, the polypeptide is characterised by an antibacterial activity against Gram-negative bacteria. The Gram-negative bacteria may be of the Enterobacteriaceae family. In some embodiments, the polypeptide is characterised by an antibacterial activity against drug-resistant bacteria. In some embodiments, the polypeptide shows antibacterial activity against *Escherichia coli, Klebsiella pneumonia, Morganella morganii, Pseudomonas aeruginosa, Acinetobacter baumanii, Enterobacter cloacae, Salmonella typhimurium, Salmonella entereditis, Shigella flexneri,* or a combination thereof. In some embodiments, the polypeptide shows antibacterial activity against *Escherichia coli, Klebsiella pneumonia, Enterobacter cloacae, Salmonella typhimurium, Salmonella entereditis, Shigella flexneri,* or a combination thereof.

It is believed that the varying activities of the peptides is due to different affinities to target proteins.

In some embodiments, the polypeptide is characterised by a minimal inhibitory concentration (MIC) of about 2 μg/mL to about 10 μg/mL. In other embodiments, the MIC is less than about 90 μg/mL, about 80 μg/mL, about 70 μg/mL, about 60 μg/mL, about 50 μg/mL, or about 40 μg/mL.

In some embodiments, the polypeptide is an isolated polypeptide. "Isolated polypeptide" refers to a polypeptide which is substantially separated from other contaminants that naturally accompany it, e.g., protein, lipids, and polynucleotides. The term embraces

polypeptides which have been removed or purified from their naturally-occurring environment or expression system (e.g., host cell or *in vitro* synthesis). The polypeptide may be present within a cell, present in the cellular medium, or prepared in various forms, such as lysates or isolated preparations. The polypeptide is then separated from its native medium in order to form the isolated polypeptide.

In some embodiments, the polypeptide is synthetically produced. In this regard, the polypeptide can be formed via recombinant methods, phage systems, biological systems and/or via chemical synthesis. For example, solid-phase peptide synthesis can be used. The polypeptide may be synthesised by providing the corresponding nucleic acid sequence to a host cell and the polypeptide produced and modified *in vivo*.

The present invention also provides a method of producing a polypeptide in a host cell, the method comprising:
a) introducing to the host cell one or more nucleic acid molecules, the nucleic acid molecules configured to express a precursor polypeptide (A), a rSAM/SPASM maturase (B), a protease (C), a transporter (D) and a protease/transporter (E);
wherein the precursor polypeptide comprises a first three residue motif (from a N-terminus) and a second three residue motif, the first and second three residue motif optionally separated by 1 to 3 amino acid residue, and at least two C-terminus residues;
wherein the three residue motif is each represented by X_1-X_2-X_3;
wherein each X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine, an unnatural aromatic amino acid or a derivative thereof;
wherein each X_2 and X_3 are independently any amino acid residue;
wherein at least one of the two C-terminus residues is an aromatic residue;
wherein the rSAM/SPASM maturase (B) is capable of modifying the precursor polypeptide (A) in the host cell to form a modified precursor polypeptide with a cyclophane moiety connecting the X_1 and X_3 residues in each motif;
wherein the protease (C), transporter (D) and protease/transporter (E) are capable of cleaving the modified precursor polypeptide from the rSAM/SPASM maturase (A) to form a cleaved modified polypeptide and exporting the cleaved modified polypeptide out from the host cell.

The nucleic acid molecule is a polynucleotide. In some embodiments, at least the nucleic acid molecule configured to express the precursor polypeptide (A) is derived from a Xye

species. In some embodiments, at least the nucleic acid molecule configured to express the precursor polypeptide (A) and the nucleic acid molecule configured to express the rSAM/SPASM maturase (B) is derived from a Xye species.

In some embodiments, the nucleic acid molecule configured to express the precursor polypeptide (A) is from one Xye species while the nucleic acid molecules configured to express the rSAM/SPASM maturase (B), the protease (C), the transporter (D) and the protease/transporter (E) are from another Xye species. In some embodiments, the nucleic acid molecule configured to express the rSAM/SPASM maturase (B) is from one Xye species while the nucleic acid molecules configured to express the precursor polypeptide (A), the protease (C), the transporter (D) and the protease/transporter (E) are from another Xye species. In some embodiments, the nucleic acid molecule configured to express the protease (C) is from one Xye species while the nucleic acid molecules configured to express the precursor polypeptide (A), the rSAM/SPASM maturase (B), the transporter (D) and the protease/transporter (E) are from another Xye species. In some embodiments, the nucleic acid molecules configured to express the transporter (D) is from one Xye species while the nucleic acid molecules configured to express the precursor polypeptide (A), the rSAM/SPASM maturase (B), the protease (C), and the protease/transporter (E) are from another Xye species. In some embodiments, the nucleic acid molecules configured to express the protease/transporter (E) is from one Xye species while the nucleic acid molecules configured to express the precursor polypeptide (A), the rSAM/SPASM maturase (B), the protease (C), and the transporter (D) are from another Xye species. In some embodiments, the nucleic acid molecules configured to express the precursor polypeptide (A) and the rSAM/SPASM maturase (B) are from one Xye species while the nucleic acid molecules configured to express the protease (C), the transporter (D) and the protease/transporter (E) are from another Xye species. In some embodiments, the nucleic acid molecules configured to express the precursor polypeptide (A), the rSAM/SPASM maturase (B), the protease (C), the transporter (D) and the protease/transporter (E) are from one Xye species.

In some embodiments, the nucleic acid molecule is derived from a *Xenorhabdus*, *Yersinia* and *Erwinia* (Xye) maturase system. The Xye maturase system is named after three bacterial genera where it is commonly found: Xenorhabdus, Yersinia, and Erwinia, but also includes other bacterial genus where it may also be found, such as Serratia and Photorhabdus. In some embodiments, the nucleic acid molecule configured to express

the precursor polypeptide is derived from a bacterial species selected from *Serratia marcescens (smc)*, *Erwinia toletana (etc)*, *Photorhabdus australis (pac) or Xenorhabdus nematophila (xnc).* In some embodiments, the nucleic acid molecule configured to express the rSAM/SPASM maturase is derived from a bacterial species selected from *Serratia marcescens (smc)*, *Erwinia toletana (etc)*, *Photorhabdus australis (pac) or Xenorhabdus nematophila (xnc).* In some embodiments, the nucleic acid molecule configured to express the protease, transporter and protease/transporter are derived from *Xenorhabdus nematophila (xnc).*

In some embodiments, the nucleic acid molecules configured to express the precursor polypeptide is derived from a bacterial species selected from *Xenorhabdus griffiniae VH1 (xgc), Pandoraea sp. PE-S2R-1 (psc), Pandoraea oxalativorans DSM 23570 (poc), Photorhabdus heterorhabditis Q614 (phc), Kosakonia cowanii pasteuri (kcc2 and kcc1kcc1), Bordetella bronchialis AU17976 (bbc)* and *Photorhabdus laumondii BOJ-47 (plc)*.

In some embodiments, only the nucleic acid molecules configured to express protease, transporter and protease/transporter are derived from *Xenorhabdus Spp.*

The nucleic acid molecules may each individually express a precursor polypeptide, a rSAM/SPASM maturase, a protease, a transporter and a protease/transporter. Alternatively, the nucleic acid molecules may be fused. In other words, the nucleic acid molecules are operably linked to a first promoter; i.e. the nucleic acid molecules are part of one expression unit. In some embodiments, at least the nucleic acid molecule expressing the protease, the nucleic acid molecule expressing the transporter and the nucleic acid molecule expressing the protease/transporter are fused. In some embodiments, the nucleic acid molecule expressing the precursor polypeptide and the nucleic acid molecule expressing the rSAM/SPASM maturase are fused. In some embodiments, the nucleic acid molecule expressing the rSAM/SPASM maturase, the nucleic acid molecule expressing the protease, the nucleic acid molecule expressing the transporter and the nucleic acid molecule expressing the protease/transporter are fused. In some embodiments, the nucleic acid molecule expressing the precursor polypeptide, the nucleic acid molecule expressing the rSAM/SPASM maturase, the nucleic acid molecule expressing the protease, the nucleic acid molecule expressing the transporter and the nucleic acid molecule expressing the protease/transporter are

fused.

In some embodiments, the nucleic acid molecule expressing the precursor polypeptide and the nucleic acid molecule expressing the rSAM/SPASM maturase are fused or operably linked to a first promoter, and the nucleic acid molecule expressing the protease, the nucleic acid molecule expressing the transporter and the nucleic acid molecule expressing the protease/transporter are fused or operably linked to a second promoter.

In some embodiments, the nucleic acid molecule expressing the precursor polypeptide is operably linked to a first promoter, and the nucleic acid molecule expressing the rSAM/SPASM maturase, the nucleic acid molecule expressing the protease, the nucleic acid molecule expressing the transporter and the nucleic acid molecule expressing the protease/transporter are fused or operably linked to a second promoter.

When the nucleic acid molecules are fused or linked, they may be fused in any order. For example, the nucleic acid molecule expressing the precursor polypeptide (A), the nucleic acid molecule expressing the rSAM/SPASM maturase (B), the nucleic acid molecule expressing the protease (C), the nucleic acid molecule expressing the transporter (D) and the nucleic acid molecule expressing the protease/transporter (E) may be fused as BACDE, BADEC, BAECD, BADCE, BACED, BAEDC, ABCDE, ABDEC, ABECD, ABDCE, ABCED, or ABEDC. When C, D and E are fused, they may be fused as CDE, DEC, ECD, DCE, CED, or EDC. When A and B are fused, they may be fused as AB or BA.

In some embodiments, at least one motif comprises X_1 and X_3 connected via phenylene to form a cyclophane moiety. In some embodiments, at least one motif comprises X_1 and X_3 connected via indolylene to form a cyclophane moiety. In some embodiments, the two motifs separately comprises phenylene and indolylene.

The present invention also provides a method of producing a polypeptide in a host cell, the method comprising:
a) introducing to the host cell one or more nucleic acid molecules, the nucleic acid molecules configured to express a precursor polypeptide, a rSAM/SPASM maturase, a protease, a transporter and a protease/transporter;

wherein the precursor polypeptide comprises a first three residue motif (from a N-terminus) and a second three residue motif, the first and second three residue motif optionally separated by 1 to 3 amino acid residue, and at least two C-terminus residues;
wherein the three residue motif is each represented by X_1-X_2-X_3;
wherein each X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine, or an unnatural aromatic amino acid residue;
wherein each X_2 and X_3 are independently any amino acid residue;
wherein at least one of the two C-terminus residues is an aromatic residue;
wherein the rSAM/SPASM maturase is capable of modifying the precursor polypeptide in the host cell to form a modified precursor polypeptide with a cyclophane moiety connecting the X_1 and X_3 residues in each motif;
wherein X_1 and X_3 in the second motif are connected via phenylene to form a cyclophane moiety;
wherein only the protease, transporter and protease/transporter are derived from *Xenorhabdus Spp;*
wherein the protease, transporter and protease/transporter are capable of cleaving the modified precursor polypeptide from the rSAM/SPASM maturase to form a cleaved modified polypeptide and exporting the cleaved modified polypeptide out from the host cell.

The terms "host", "host cell", "host cell line" and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells", which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein. A host cell is any type of cellular system that can be used to synthesis a modified polypeptide of the present invention. Host cells include cultured cells, e.g., mammalian cultured cells, such as CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, yeast cells, insect cells, and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue.

In some embodiments, the method further comprises a step of culturing the host cell under conditions suitable for the production of the polypeptide.

The precursor polypeptide may be of any sequence length, as long as it comprises at least two of the three residue motif optionally separated by 1 to 3 amino acid residue and at least two C-terminus residues. The precursor polypeptide, which does not comprise a cyclophane, is then modified by the rSAM/SPASM maturase to form a cyclophane containing modified precursor polypeptide. The modified precursor polypeptide may then be cleaved and transported out from the host cell by the protease, transporter and protease/transporter.

In some embodiments, the precursor polypeptide or the nucleic acid molecule configured to express the precursor polypeptide is derived from a bacterial strain as shown in Table 3. In some embodiments, the precursor polypeptide or the nucleic acid molecule configured to express the precursor polypeptide is derived from *Serratia marcescens (smc)*, *Erwinia toletana (etc)*, *Photorhabdus australis (pac), Xenorhabdus nematophila (xnc), Xenorhabdus griffiniae VH1 (xgc), Pandoraea sp. PE-S2R-1 (psc), Pandoraea oxalativorans DSM 23570 (poc), Photorhabdus heterorhabditis Q614 (phc), Kosakonia cowanii pasteuri (kcc2 and kcc1), Bordetella bronchialis AU17976 (bbc)* or *Photorhabdus laumondii BOJ-47 (plc).*

The precursor polypeptide and the rSAM/SPASM maturase (or the nucleic acid molecule configured to express the precursor polypeptide and rSAM/SPASM maturase) may be derived from the same bacterial strain, or may be of different bacterial strains. In some embodiments, the precursor polypeptide and rSAM/SPASM maturase (or the nucleic acid molecule configured to express the precursor polypeptide and rSAM/SPASM maturase) are derived from a bacterial strain as shown in Table 3. In some embodiments, the precursor polypeptide is fused to the rSAM/SPASM maturase. In some embodiments, the precursor polypeptide are transcribed and translated separately from the rSAM/SPASM maturase.

The amino acid sequence of the precursor polypeptide may be at least 70% identical to the amino acid sequence of of SEQ ID NO: [XyeA] (see Table 4 below). The amino acid sequence of the precursor polypeptide may be at least 70% identical to the amino acid sequence of SEQ ID NO: [SmcA], SEQ ID NO: [EtcA], SEQ ID NO: [PacA], SEQ ID NO:

[XgcA], SEQ ID NO: [PscA], SEQ ID NO: [PocA], SEQ ID NO: [PhcA], SEQ ID NO: [Kcc2A] SEQ ID NO: Kcc1A, SEQ ID NO: [BbcA] or SEQ ID NO: [PlcA].

The amino acid sequence of the rSAM/SPASM maturase may be at least 70% identical to the amino acid sequence of of SEQ ID NO: [XyeB] (see Table 4 below).

The term "rSAM" refers to radical S-adenosylmethionine. The rSAM enzyme may be an rSAM enzyme of the *Xenorhabdus*, *Yersinia* and *Erwinia* (XYE) maturase system (Xye, TIGR04496, IPR030989), Glycine-rich repeat (Grr) maturase system (GrrM, TIGR04261, IPR026357) or the Fxs maturase system (FxsB, TIGR04269, IPR026335). In some embodiments, the rSAM/SPASM maturase is from a *Xenorhabdus*, *Yersinia* and *Erwinia* (XYE) maturase system.

The rSAM enzyme may also be an enzymatically active fragment of an rSAM enzyme of the *Xenorhabdus*, *Yersinia* and *Erwinia* (XYE) maturase system (XyeB, TIGR04496, IPR030989), Glycine-rich repeat (Grr) maturase system (GrrM, TIGR04261, IPR026357) or the Fxs maturase system (FxsB, TIGR04269, IPR026335). In some embodiments, the rSAM/SPASM maturase is an enzymatically active fragment from a *Xenorhabdus*, *Yersinia* and *Erwinia* (XYE) maturase system.

The rSAM enzyme may have an amino acid sequence that is at least 70% (or 75%, 80%, 85%, 90% or 95%) identical to the following sequences:

XncB (*Xenorhabdus nematophila)*:
MTTSKSEKIKHLEIILKISERCNINCSYCYVFNMGNSLATDSPPVISLDNVLALRGFFERSAAENEI
EVIQVDFHGGEPLMMKKDRFDQMCDILRQGDYSGSRLELALQTNGILIDDEWISLFEKHKVHASI
SIDGPKHINDRYRLDRKGKSTYEGTIHGLRMLQNAWKQGRLPGEPGILSVANPTANGAEIYHHFA
NVLKCQHFDFLIPDAHHDDDIDGIGIGRFMNEALDAWFADGRSEIFVRIFNTYLGTMLSNQFYRV
IGMSANVESAYAFTVTADGLLRIDDTLRSTSDEIFNAIGHLSELSLSGVLNSPNVKEYLSLNSELPS
DCADCVWNKICHGGRLVNRFSRANRFNNKTVFCSSMRLFLSRAASHLITAGIDEETIMKNIQK
(SEQ ID NO: 61)

YkcB (*Yersinia kristensenii*):
MEVITGSEGRVMLNLLIEKNIRHLEIILKISERCNINCDYCYVFNKGNSAADDSPARLSNKNIHHLV
CFLQRACQEYKIGTVQIDFHGGEPLLMKKENFTDMCIQLISGNYCGSNIRLALQTNATLIDNEWIA

IFEKYSVNVSISIDGPKHINDRHRLDTKGRSTYESTVRGLRILQNAYQQGRLPSDPGILCVTNAQA
NGAEIYRHFVDELGVYSFDFLIPDDSYKDAHPDAVGIGRFLNEALDEWVKDNNAKIFVRLFQTHIA
SLLGQKNSGVLGHTPNITGVYALTVSSDGFVRVDDTLRSTSDRMFNPIGHLSEVNLSNVFASPQF
QEYSSIGQSLPTECEGCIWENICAGGRIVNRFSTEDRFKHKSIYCYSMRTFLSRSSAHLLNMGIKE
ERIMAAIRA
(SEQ ID NO: 62)

EtcB (*Erwinia toletana)*:
MTQLKGEKIKHLEIILKISERCNINCTYCYVFNMGNTLATDSTPVISLDNVYALRGFFERSAAENDI
EVIQVDFHGGEPLMMKKDRFDRMCQILLQGNYRSSKFELALQTNGILIDDEWIALFEKHQVHASI
SVDGPKHINDRHRLDRKGKSTYEGTITGLRLLQNAWQQGRLPGEPGILSVANANANGAEIYRHF
ADTLQCQRFDFLIPDDHHDDSPDGEGVGRFLNEALDAWFADGRPEIFIRIFNTYLGTMLNSQFNR
VLGMSANVESAYAFTVTADGMLRIDDTLRSTSDEIFNAVGHVSELSLARVLETSCVKEYLALSSNL
PTVCAECVWNNICHGGRLVNRFSRTNRFNNKTVFCKSMRLFLSRAASHLMASGVDEKEIMKNIQ
K
(SEQ ID NO: 63)

MscB (Micromonospora sp.):
MAPGPARAALTEFVLKVHARCDLACDHCYVYEHADQSWRRRPVRMTPEVLRTAAGRIAEHAAAH
DLPDVTVILHGGEPLLLGAERLGEVLADLRRVIDPVTRLRLGMQTNGVLLSERLCDLLAEHDVAVG
VSLDGDRAANDRHRRFRSGAGSYDQVLRAIGLLRRPAYRRIYSGLLCTVDVRNDPIAVYESLLTQ
EPPRIDFLLPHATWDDPPWRPAGGGTAYAGWLRAVYDRWLADGRPVSVRLFDSLLSTAAGGPS
GTEWLGLDPVDLAVVETDGEWEQADSLKTAYDGAPATGMTVFSHAADDVAASPLLARRRSGRA
GLSDECRRCPVVDQCGGGLFAHRYGAGHFDHPSVYCADLKELIVHVNENPPAPVRLDAGLPDDF
IDRLAALTGDRVAIGRLVEAQIAIVRALLAEVADRLPAGGAGADGWEALTALDRSAPESVARIAAH
PYVRAWAVDCLAGSGTGARQGPDYLSALAVAAALDAGTPVRLDVPVRSGRLHLPTVGTVLLPEV
GDGAARVETGPGSLRVAAGDVTVAIRPGTPGDAPRWWPTRVLAAPDVSVLLEDGDPHRDCHRL
PAGDRLDDAGAARWAETFAAAWQVIRDEVPGHAEELRAGLRAVVPLRRSGAGVSEASTARQAF
GGVAATETDAGSLAVLLVHEFQHSKMNALLDICDLVDGTRPIDITVGWRPDPRPAEAVLHGIYAH
AAVADIWRIRADRQVDGAQAVYRRYRDWTAEAIGALQRADALTPAGSRLVRQVARSMSGWPS
(SEQ ID NO: 64)

OscB (Oscillatoriales cyanobacterium):
MINPTLLNPEKIDISKFGPINLVVIQATSFCNLNCDYCYLPNRDLKNTLSLDLIEPIFKNIFNSPFVG
DEFTICWHAGEPLAVPISFYESAFQLIQAADQKYNQKQAKIWHSVQTNATYINQKWCDFIQEHNI

CVGVSLDGPEFIHDAHRQTRKGTGSHAQTMRGISFLQKNNIPFYVISVVTQDSLNYADEIFNFFR
ENGIYDVGFNLEEIEGVNQSSTLEAVGTSEKYRAFMQRFWELTSEVQGEFNLREFEAICGLIYSNT
RLTQTDMNNPFVLINIDYQGNFSTFDPELLSVNIKPYGNFILGNVLTDSFESVCDTEKFQKIYTDM
QEGIKLCRETCEYFGVCGGGAGSNKYWENGTFACSETMACRYRIKVVTDIILDKLENSLGLVENC
(SEQ ID NO: 65)

LscB (Lyngbya sp.):
MTISKMNLPVQTDNFRASSTLDLSAFGPINLVVIQSTSFCNLNCDYCYLRDRQSKNRLSLDLIEPIL
KTVLTSPFVGCDFTILWHAGEPLAMPISFYDSATALIREAERQYKTQPIQIFQSIQTNATLINQAWC
DCFRRNEIYVGVSLDGPAFLHDAHRQTYKGTGTHAATMRGISLLQKNEIPFNVICVLTQDSLDYP
DEIFNFFRSNRITEVGFNMEEAEGVHQHSTLDQQGTEERYRAFMQRFWDLTVQAKGEFKLREFE
TICTLAYTGDRLGYTDMNQPFVIVNFDHQGNFSTFDPELLSFKIKEYGDFVLGNVLHNTLESVCQT
EKFQKIYQDMAAGVVQCRQSCEYFGLCGGGAGSNKYWENGTFNCTETKACRYRIKVIADIVLEG
LENSLELANSIS
(SEQ ID NO: 66)

GscB (Geminocytis sp.):
MSIVTSKPVINFKNTANFGPISLIIIQPNSFCNLDCDYCYLPDRHLQNKLSLDLIDPIFKSIFTSPFLG
CDFGVCWHAGEPLTMPVSFYKSAFQLIEEANTKYNKSEYSFYHSYQTNGTLINQGWCDLWQEYP
VHVGVSIDGPAFLHDVHRKNRKGGNSHDLTMRGIRYLQKNNIPYNTISVITEESLNYPDEMFNFF
AENEIYDLAFNMEETEGVNELTSLNGIEIEHKYSQFIKRFWQLVTESKLPFIVREFEILISLIYSGNR
LTNTDMNKPFVIVNFDYQGNFSTFDPELLSVKTDKYGDFIFGNVLKDSLESICETEKFKTIYKDIND
GVKLCSDNCSYFGICGGGAGSNKYWENGTFASMETQACRYRIKILTDVLVSTIENSLGL
(SEQ ID NO: 67)

In one embodiment, the rSAM enzyme is a C-terminal truncated MscB-375 enzyme with the following sequence:

MAPGPARAALTEFVLKVHARCDLACDHCYVYEHADQSWRRRPVRMTPEVLRTAAGRIAEHAAAH
DLPDVTVILHGGEPLLLGAERLGEVLADLRRVIDPVTRLRLGMQTNGVLLSERLCDLLAEHDVAVG
VSLDGDRAANDRHRRFRSGAGSYDQVLRAIGLLRRPAYRRIYSGLLCTVDVRNDPIAVYESLLTQ
EPPRIDFLLPHATWDDPPWRPAGGGTAYAGWLRAVYDRWLADGRPVSVRLFDSLLSTAAGGPS
GTEWLGLDPVDLAVVETDGEWEQADSLKTAYDGAPATGMTVFSHAADDVAASPLLARRRSGRA
GLSDECRRCPVVDQCGGGLFAHRYGAGHFDHPSVYCADLKELIVHVNENPPAPV
(SEQ ID NO: 68).

The enzymes as referred to herein may comprise one or more conservative amino acid substitution.

In one embodiment, the rSAM enzyme is an enzymatically active fragment of any one of the above sequences. In one embodiment, the enzymatically active fragment is one that comprises the rSAM and SPASM domains (such as CNINCSYC (SEQ ID NO: 69) and CADCVWNKIC (SEQ ID NO: 70) in XncB). In one embodiment, the enzymatically active fragment is from YkcB, wherein the rSAM domain is CNINCDYCYVFNK (SEQ ID NO: 213) and the SPASM domain is CEGCIWENIC (SEQ ID NO: 214). In one embodiment, the enzymatically active fragment is from EtcB, wherein the rSAM domain is CNINCTYC (SEQ ID NO: 215), and the SPASM domain is CAECVWNNIC (SEQ ID NO: 216). In one embodiment, the enzymatically active fragment is from MscB, wherein the rSAM domain is CDLACDHC (SEQ ID NO: 217), and the SPASM domain is CRRCPVVDQC (SEQ ID NO: 218). In one embodiment, the enzymatically active fragment is from OscB, wherein the rSAM domain is CNLNCDYC (SEQ ID NO: 219), and the SPASM domain is CRETCEYFGVC (SEQ ID NO: 220). In one embodiment, the enzymatically active fragment is from LscB, wherein the rSAM domain is CNLNCDYC (SEQ ID NO: 221), and the SPASM domain is CRQSCEYFGLC (SEQ ID NO: 222). In one embodiment, the enzymatically active fragment is from GscB, wherein the rSAM domain is CNLDCDYC (SEQ ID NO: 223), and the SPASM domain is CSDNCSYFGIC (SEQ ID NO: 224).

The rSAM enzyme may be a XyeB, GrrM or FxsB rSAM enzyme from a bacterial genus listed in Tables 4-6.

Table 4. Precursor (XyeA, IPR030990) and rSS (XyeB, IPR030989) paired sequences from the UniProt database.

Accession No. Precursor (XyeA)	Accession No. rSS (XyeB)	Strain
A0A1C0TZE6	A0A1C0TZL9	*Photorhabdus australis*
A0A1Q4P361	A0A1Q4P3B6	*Serratia marcescens*
A0A084A5U2	A0A084A5U1	*Serratia* sp. DD3
A0A0B6XF00	A0A0B6XFQ9	*Xenorhabdus bovienii*
A0A077P0J4	A0A077P0L0	*Xenorhabdus bovienii* str. oregonense
A0A1I5BFB3	A0A1I5BES0	*Xenorhabdus japonica*
D3VF66	D3VF67	*Xenorhabdus nematophila* (strain ATCC 19061 / DSM 3370 / LMG 1036 /NCIB 9965 / AN6)
A0A0R4D012	A0A0R4D0A6	*Xenorhabdus nematophila* AN6/1
N1NN13	N1NM08	*Xenorhabdus nematophila* F1

A0A0A8NQW6	A0A0A8NMB7	*Xenorhabdus nematophila* str. Websteri
A0A2D0KYU9	A0A2D0KZ85	*Xenorhabdus* sp. KJ12.1
A0A2D0K7T4	A0A2D0K7L0	*Xenorhabdus* sp. KK7.4
A0A2D0KQ63	A0A2D0KQJ1	*Xenorhabdus stockiae*
A0A2G4TZ16	A0A2G4TZ87	*Yersinia bercovieri*
A0A0E1NG59	A0A0E1NDZ2	*Yersinia enterocolitica*
A0A0T7NPU9	A0A0T7NP34	*Yersinia enterocolitica*
A0A0H3NSR9	A0A0H3NRG2	*Yersinia enterocolitica* subsp. palearctica serotype O:3 (strain DSM 13030 / CIP 106945 / Y11)
F4MYR4	F4MYR5	*Yersinia enterocolitica* W22703
A0A209AZF0	A0A209AZP3	*Yersinia frederiksenii*
A0A0T9N5M4	A0A0T9N4P3	*Yersinia kristensenii*
A0A0T9U1K9	A0A0T9U1I2	*Yersinia kristensenii*
A0A0U1HZP4	A0A0U1HZK1	*Yersinia mollaretii*
C4S8Z7	C4S8Z6	*Yersinia mollaretii* ATCC 43969

Table 5. Precursor (GrrA, IPR026356) and rSS (GrrM, IPR026357) paired sequences from the UniProt database.

Accession No. Precursor (GrrA)	Accession No. rSAM (GrrM)	Strain
A0A1Q3KH01	A0A1Q3KH56	*Alphaproteobacteria bacterium* 65-37
A0A2T1F2L2	A0A2T1F2I9	*Aphanothece* cf. *minutissima* CCALA 015
A0A2T1LXR5	A0A2T1LXR7	*Aphanothece hegewaldii* CCALA 016
G5J0Q7	G5J0Q8	*Crocosphaera watsonii* WH 0003
G5J8Q7	G5J0Q8	*Crocosphaera watsonii* WH 0003
G5J8Q8	G5J0Q8	*Crocosphaera watsonii* WH 0003
T2IXQ8	T2IYC6	*Crocosphaera watsonii* WH 0005
T2IXZ4	T2IYC6	*Crocosphaera watsonii* WH 0005
T2J085	T2IYC6	*Crocosphaera watsonii* WH 0005
T2JXQ3	T2JW16	*Crocosphaera watsonii* WH 0402
T2JY88	T2JW16	*Crocosphaera watsonii* WH 0402
T2JZD7	T2JW16	*Crocosphaera watsonii* WH 0402
Q4BWP4	Q4BWP2	*Crocosphaera watsonii* WH 8501
A0A1Z9JEB4	A0A1Z9JEI5	*Cyanobacteria bacterium* TMED177
A0A1Z9JES1	A0A1Z9JEI5	*Cyanobacteria bacterium* TMED177
A0A1Z9JIL3	A0A1Z9JEI5	*Cyanobacteria bacterium* TMED177
A0A1Z9LF09	A0A1Z9LEY5	*Cyanobacteria bacterium* TMED188
A0A1Z9LF10	A0A1Z9LEY5	*Cyanobacteria bacterium* TMED188
K9Z5N8	K9Z3I9	*Cyanobacterium aponinum* (strain PCC 10605)
A0A2G3PAN6	A0A2G3P8V3	*Cyanobacterium aponinum* IPPAS B-1201
K9PAE0	K9PBG1	*Cyanobium gracile* (strain ATCC 27147 / PCC 6307)
A0A2W6YZ82	A0A2W6YZU4	*Cyanobium* sp
A0A2W6ZHA8	A0A2W7A6G1	*Cyanobium* sp
A0A326QHT4	A0A326QDC6	*Cyanobium* sp
A0A2D6FEB5	A0A2D6FEG4	*Cyanobium* sp. ARS6
A0A081GHK6	A0A081GHK5	*Cyanobium* sp. CACIAM 14
A0A2E1IN00	A0A2E1IQ77	*Cyanobium* sp. MED843

A0A2E1IQ42	A0A2E1IQ77	*Cyanobium* sp. MED843
A0A2E1IQ50	A0A2E1IQ77	*Cyanobium* sp. MED843
A0A2E0AN10	A0A2E0AMN8	*Cyanobium* sp. NAT70
A0A182AQN3	A0A182ASF1	*Cyanobium* sp. NIES-981
A0A182AU27	A0A182ASU9	*Cyanobium* sp. NIES-981
B5IK36	B5IK37	*Cyanobium* sp. PCC 7001
B5ILU6	B5ILU5	*Cyanobium* sp. PCC 7001
A0A2E4LLZ3	A0A2E4LLZ4	*Cyanobium* sp. SAT1300
A0A2P7MTB4	A0A2P7MT91	*Cyanobium usitatum* str. Tous
B1X121	B1X120	*Cyanothece* sp. (strain ATCC 51142)
B1X122	B1X120	*Cyanothece* sp. (strain ATCC 51142)
B7KDY1	B7KDY3	*Cyanothece* sp. (strain PCC 7424)
B7KDY2	B7KDY3	*Cyanothece* sp. (strain PCC 7424)
B8HSH4	B8HSH5	*Cyanothece* sp. (strain PCC 7425 / ATCC 29141)
B8HSH8	B8HSH9	*Cyanothece* sp. (strain PCC 7425 / ATCC 29141)
B8HV48	B8HUF3	*Cyanothece* sp. (strain PCC 7425 / ATCC 29141)
E0UHF6	E0UHF5	*Cyanothece* sp. (strain PCC 7822)
E0UHF7	E0UHF5	*Cyanothece* sp. (strain PCC 7822)
B7JUH9	B7JUI0	*Cyanothece* sp. (strain PCC 8801)
A3INK4	A3INK3	*Cyanothece* sp. CCY0110
A3INK5	A3INK3	*Cyanothece* sp. CCY0110
A0A3B8XXV7	A0A3B8Y1T1	*Cyanothece* sp. UBA12306
A0A3B8XZG8	A0A3B8Y6Z2	*Cyanothece* sp. UBA12306
A0A3B8Y4Z1	A0A3B8Y1T1	*Cyanothece* sp. UBA12306
A0A1T4RKP1	A0A1T4RK36	*Enhydrobacter aerosaccus*
A0A2P8W4T2	A0A2P8W4T3	*filamentous cyanobacterium* CCT1
A0A0D6AAG1	A0A0D6AAL6	*Geminocystis* sp. NIES-3708
A0A0D6AAQ5	A0A0D6AAL6	*Geminocystis* sp. NIES-3708
A0A0D6AVA7	A0A0D6AVB2	*Geminocystis* sp. NIES-3709
A0A0D6AWJ4	A0A0D6AVB2	*Geminocystis* sp. NIES-3709
A0A261KMH7	A0A261KM11	*Hydrocoleum* sp. CS-953
A0A261KMK1	A0A261KM12	*Hydrocoleum* sp. CS-953
A0A261KPG0	A0A261KM13	*Hydrocoleum* sp. CS-953
A0A1L3EWS6	A0A1L3EWP1	*Luteibacter rhizovicinus* DSM 16549
A0A2T5LGC6	A0A2T5LG77	*Luteibacter* sp. OK325
A0YYD0	A0YYD1	*Lyngbya* sp. (strain PCC 8106)
A0A1I3WAQ4	A0A1I3WAK9	*Methylocapsa palsarum*
A0A2J7TE77	A0A2J7TE75	*Methylocella silvestris*
B8EQ29	B8EQ28	*Methylocella silvestris* (strain DSM 15510 / CIP 108128 / LMG 27833 /NCIMB 13906 / BL2)
A0A3E0LTQ3	A0A2W4QF24	*Microcystis aeruginosa* DA14
L8NY47	A0A2W6YZU4	*Microcystis aeruginosa* DIANCHI905
A0A3N0WKD4	A0A2W7B0M0	*Microcystis aeruginosa* FACHB-524
A0A1V4BUU7	A0A2Z6UYG4	*Microcystis aeruginosa* KW
A0A0F6RM21	A0A3E0LNV2	*Microcystis aeruginosa* NIES-2549
A0A2H6BTD4	A0A3E0LRP7	*Microcystis aeruginosa* NIES-298
A0A0A1VYH5	A0A3N0VP57	*Microcystis aeruginosa* NIES-44

A0A2H6KZG4	A0A3N5J195	*Microcystis aeruginosa* NIES-87
A0A139GHJ6	A0A3R7P7F6	*Microcystis aeruginosa* NIES-88
A0A1E4QIR2	A0A3S1IS64	*Microcystis aeruginosa* NIES-98
A8YAG5	A0A3S3KC59	*Microcystis aeruginosa* PCC 7806
I4GMR0	A0A402AY08	*Microcystis aeruginosa* PCC 7941
I4FZ11	A0A402DGT7	*Microcystis aeruginosa* PCC 9443
I4IUU0	A0A402DKN0	*Microcystis aeruginosa* PCC 9701
I4FU32	A0A429FKD6	*Microcystis aeruginosa* PCC 9717
I4GVW3	A0A495Q9Z9	*Microcystis aeruginosa* PCC 9806
I4HD64	A0A4P5VFP0	*Microcystis aeruginosa* PCC 9807
I4HZK0	A0A4P5VNH3	*Microcystis aeruginosa* PCC 9808
I4HQP4	A0A4P5Z922	*Microcystis aeruginosa* PCC 9809
A0A2Z6UMP5	A0A4P6JJ41	*Microcystis aeruginosa* Sj
S3JFW1	A0A4P6JTC0	*Microcystis aeruginosa* SPC777
A0A3E0LWL6	A0A4P6LF79	*Microcystis aeruginosa* TA09
L7E5P1	A0A4P7ZWF9	*Microcystis aeruginosa* TAIHU98
A0A3E0LEJ9	A0A4Q0QKH8	*Microcystis flos-aquae* DF17
A0A3E0L6T7	A0A4R2MAC4	*Microcystis flos-aquae* TF09
A0A0K1S6M0	A0A4V0YR58	*Microcystis panniformis* FACHB-1757
A0A2L2XVF6	A0A510PMW7	*Microcystis* sp. 0824
A0A2P1UF64	A0A521QRV3	*Microcystis* sp. MC19
I4IH33	A0A525JRG1	*Microcystis* sp. T1-4
A0A3G9JV83	A0A537IV48	*Microcystis viridis* NIES-102
A0A3E0LNP2	A0A537WMI1	*Microcystis wesenbergii* TW10
A0A098TGT4	A0A098TIF4	*Neosynechococcus sphagnicola* sy1
A0A1J5GLC7	A0A1J5G9T5	*Oscillatoriales cyanobacterium* CG2_30_40_61
A0A1J5GNK8	A0A1J5G9T5	*Oscillatoriales cyanobacterium* CG2_30_40_61
A0A2D5W495	A0A2D5W441	*Pedosphaera* sp
A0A1U7IQQ0	A0A1U7IR09	*Phormidium ambiguum* IAM M-71
A0A1J1JHQ4	A0A1J1JKY7	*Planktothrix agardhii*
A0A2Z6CEF9	A0A2Z6CEN3	*Planktothrix agardhii* NIES-204
A0A073CC77	A0A073CPJ3	*Planktothrix agardhii* NIVA-CYA 126/8
A0A1J1K3H2	A0A1J1K5L2	*Planktothrix paucivesiculata* PCC 9631
A0A1J1K4A6	A0A1J1K5L2	*Planktothrix paucivesiculata* PCC 9631
A0A1J1L466	A0A1J1L5D0	*Planktothrix rubescens*
A0A1J1L4L1	A0A1J1L5D0	*Planktothrix rubescens*
A0A1T4ZP83	A0A1T4ZPC2	*Planktothrix* sp. PCC 11201
A0A1T4ZPR1	A0A1T4ZPC2	*Planktothrix* sp. PCC 11201
A0A354WB48	A0A354WC37	*Planktothrix* sp. UBA10369
A0A1J1LRN3	A0A1J1LPS2	*Planktothrix tepida* PCC 9214
A2C6R5	A2C6R4	*Prochlorococcus marinus* (strain MIT 9303)
A2C6R6	A2C6R4	*Prochlorococcus marinus* (strain MIT 9303)
Q7TUR4	Q7V5N2	*Prochlorococcus marinus* (strain MIT 9313)
Q7V5N3	Q7V5N2	*Prochlorococcus marinus* (strain MIT 9313)
A0A163MAY1	A0A163MB05	*Prochlorococcus marinus* str. MIT 1318

A0A163MAY9	A0A163MB05	*Prochlorococcus marinus* str. MIT 1318
A0A163UYZ9	A0A163UYY0	*Prochlorococcus marinus* str. MIT 1342
A0A163UZ11	A0A163UYY0	*Prochlorococcus marinus* str. MIT 1342
A0A0A2CVT9	A0A0A2CSU8	*Prochlorococcus* sp. MIT 0701
A0A163G309	A0A163G301	*Prochlorococcus* sp. MIT 1303
A0A163G370	A0A163G301	*Prochlorococcus* sp. MIT 1303
A0A163CFK3	A0A162EHT7	*Prochlorococcus* sp. MIT 1306
A0A163CFM9	A0A162EHT7	*Prochlorococcus* sp. MIT 1306
A0A2W7AW46	A0A2W7AZA2	*Pseudanabaena* sp
A0A2W7BIW5	A0A2W7AZA2	*Pseudanabaena* sp
A0A1Q3UQZ1	A0A1Q3URB4	*Rhodospirillales bacterium* 69-11
A0A1H8W476	A0A1H8W4C7	*Rhodospirillales bacterium* URHD0017
U5D711	U5DGM8	*Rubidibacter lacunae* KORDI 51-2
A0A2T6CYV8	A0A2T6CYW6	*Spartobacteria bacterium* LR76
A0A140K7I6	A0A140K7I7	*Stanieria* sp. NIES-3757
A0A354AYF2	A0A354AYF1	*Synechococcales bacterium* UBA10510
K9RV97	K9RVS0	*Synechococcus* sp. (strain ATCC 27167 / PCC 6312)
K9RWD4	K9RVS0	*Synechococcus* sp. (strain ATCC 27167 / PCC 6312)
Q0I7K8	Q0I7K7	*Synechococcus* sp. (strain CC9311)
Q3AHW8	Q3AHW7	*Synechococcus* sp. (strain CC9605)
Q3AZB1	Q3AZB2	*Synechococcus* sp. (strain CC9902)
A5GNI4	A5GNI5	*Synechococcus* sp. (strain WH7803)
A4CQZ9	A4CQZ8	*Synechococcus* sp. (strain WH7805)
A4CR02	A4CQZ8	*Synechococcus* sp. (strain WH7805)
A0A0H4BED4	A0A0H4B9G9	*Synechococcus* sp. (strain WH8020)
Q7U8L1	Q7U8L2	*Synechococcus* sp. (strain WH8102)
A0A0H5PPM7	A0A0H5Q5R5	*Synechococcus* sp. (strain WH8103)
A0A2D6Y6K9	A0A2D6Y6L1	*Synechococcus* sp. ARS1019
Q063T1	Q063T0	*Synechococcus* sp. BL107
A0A2D5RBM0	A0A2D5RBZ8	*Synechococcus* sp. CPC100
A0A2D4YV37	A0A2D4YV84	*Synechococcus* sp. CPC35
A0A2D8TUV2	A0A2D8TUV7	*Synechococcus* sp. EAC657
A0A076H3B2	A0A076H4I8	*Synechococcus* sp. KORDI-100
A0A076H859	A0A076H950	*Synechococcus* sp. KORDI-49
A0A076HIY6	A0A076HGM3	*Synechococcus* sp. KORDI-52
A0A2D7JF21	A0A2D7JF38	*Synechococcus* sp. MED650
A0A2D7JF48	A0A2D7JF38	*Synechococcus* sp. MED650
A0A2E1IKX8	A0A2E1IKT4	*Synechococcus* sp. MED850
A0A163XXP8	A0A163XXR0	*Synechococcus* sp. MIT S9504
A0A2E0KHR0	A0A2E0KJ42	*Synechococcus* sp. NAT40
A0A2E9IYA8	A0A2E9IY90	*Synechococcus* sp. NP17
A3Z9D0	A3Z9D6	*Synechococcus* sp. RS9917
A0A1J0P9N7	A0A1J0PAS0	*Synechococcus* sp. SynAce01
A0A1Z8P5Z3	A0A3R7P7F6	*Synechococcus* sp. TMED20
A0A1Z9MG24	A0A1Z9MG09	*Synechococcus* sp. TMED205
A0A1Z9W1Y1	A0A1Z9W225	*Synechococcus* sp. TMED90
A0A1Z9W204	A0A1Z9W225	*Synechococcus* sp. TMED90
A3YUD7	A3YUD8	*Synechococcus* sp. WH 5701
G4FNN6	G4FNN7	*Synechococcus* sp. WH 8016

A0A316JQL6	A0A316JNT0	*Synechococcus* sp. XM-24
A0A068MZG7	A0A068MZ81	*Synechocystis* sp. (strain PCC 6714)
A0A068MZS1	A0A068MZ81	*Synechocystis* sp. (strain PCC 6714)
P73641	P73639	*Synechocystis* sp. (strain PCC 6803 / Kazusa)
P73642	P73639	*Synechocystis* sp. (strain PCC 6803 / Kazusa)
A0A1G7JAL7	A0A1G7JAI1	*Terriglobus roseus*
A0A146G9H0	A0A146GA35	*Terrimicrobium sacchariphilum*
L8LYM3	L8M110	*Xenococcus* sp. PCC 7305

Table 6. Precursor (FxsA, IPR026334) and rSS (FxsB, IPR026335) paired sequences from the UniProt database.

Accession No Precursor (FxsA)	Accession No rSAM (FxsB)	Strain
A0A024YVT1	A0A024YTX8	Streptomyces sp. PCS3-D2
A0A086GKG9	A0A086GKG5	Streptomyces scabiei
A0A086H3F5	A0A086H3F6	Streptomyces scabiei
A0A0B5DCU4	A0A0B5D7B6	Streptomyces nodosus
A0A0B5DFK9	A0A0B5DGY8	Streptomyces nodosus
A0A0C2AZ32	A0A0C1XRC9	Streptomyces sp. AcH 505
A0A0C2JH84	A0A0C2FG78	Streptomonospora alba
A0A0D8BGK1	A0A0D8BE63	Frankia torreyi
A0A0F0HR20	A0A0F0HQY3	Saccharothrix sp. ST-888
A0A0F2TMH1	A0A0F2TLU9	Streptomyces rubellomurinus (strain ATCC 31215)
A0A0F2TP24	A0A0F2TK09	Streptomyces rubellomurinus (strain ATCC 31215)
A0A0F7FYW7	A0A0F7CPX4	Streptomyces xiamenensis
A0A0F7VTY0	A0A0F7VWL0	Streptomyces leeuwenhoekii
A0A0G3UPS1	A0A0G3UX52	Streptomyces sp. Mg1
A0A0H1ANZ2	A0A0H1ATT0	Streptomyces sp. KE1
A0A0L0L3D8	A0A0L0L3M2	Streptomyces stelliscabiei
A0A0L8KXY1	A0A0L8KXN5	Streptomyces resistomycificus
A0A0L8N4S2	A0A0L8N542	Streptomyces virginiae
A0A0M4DX52	A0A0M4DES0	Streptomyces pristinaespiralis
A0A0M8UJ12	A0A0M9Z7D0	Streptomyces sp. H021
A0A0M8X5P8	A0A0M8X512	Streptomyces sp. NRRL B-1140
A0A0M8Z5Z8	A0A0M8Z7D9	Streptomyces sp. NRRL F-7442
A0A0M9CUH5	A0A0M9CUQ8	Streptomyces sp. XY332
A0A0M9X8N0	A0A0M9X8Q2	Streptomyces caelestis
A0A0N0N1U5	A0A0N1GCD1	Actinobacteria bacterium OK074
A0A0N1GPU5	A0A0N1NRU5	Actinobacteria bacterium OV320
A0A0N1GVW3	A0A0N1GG97	Actinobacteria bacterium OK074
A0A0N1H1K8	A0A0N1GVW6	Actinobacteria bacterium OV450
A0A0N6ZI00	A0A0N6ZHQ7	Streptomyces sp. CCM_MD2014
A0A0Q1CC38	A0A0Q0XVU4	Frankia sp. ACN1ag
A0A0Q8P0V1	A0A0Q8P0C1	Kitasatospora sp. Root187
A0A0S1UIU0	A0A0S1UIV4	Streptomyces sp. FR-008

A0A0S4QS43	A0A0S4QR97	Frankia irregularis
A0A0T1TPK5	A0A0T1TPF8	Streptomyces sp. Root1310
A0A0U3PLY0	A0A0U3QPY8	Streptomyces sp. CdTB01
A0A0X3SAJ4	A0A0X3S963	Streptomyces sp. NRRL F-5122
A0A0X7JP05	A0A0X7JP10	Streptomyces albus subsp. albus
A0A100JQ89	A0A100JQ96	Streptomyces scabiei
A0A100JSG9	A0A100JSI9	Streptomyces scabiei
A0A100JVX7	A0A100JVX4	Streptomyces scabiei
A0A101N4D8	A0A124H9X5	Streptomyces pseudovenezuelae
A0A101SUF2	A0A124I2K5	Streptomyces bungoensis
A0A117E9F8	A0A117E9X1	Streptomyces acidiscabies
A0A126Y013	A0A126Y041	Streptomyces albidoflavus
A0A162JNC9	A0A166Q011	Frankia sp. EI5c
A0A171DNJ8	A0A171DNJ7	Planomonospora sphaerica
A0A1A8ZLD1	A0A1A8ZKQ9	Micromonospora narathiwatensis
A0A1A9CJH0	A0A1A9CLI2	Streptomyces sp. OspMP-M45
A0A1A9DPC8	A0A1A9DPD0	Streptomyces sp. Ncost-T6T-1
A0A1C4HUF9	A0A1C4HUC7	Streptomyces sp. ScaeMP-e83
A0A1C4L932	A0A1C4L9L5	Streptomyces sp. TverLS-915
A0A1C4N8D6	A0A1C4N823	Streptomyces sp. DvalAA-14
A0A1C4NZW7	A0A1C4NZD7	Streptomyces sp. BvitLS-983
A0A1C4TA70	A0A1C4T9T5	Streptomyces sp. DvalAA-43
A0A1C4TI64	A0A1C4TI12	Streptomyces sp. DfronAA-171
A0A1C4U9B9	A0A1C4U928	Micromonospora chokoriensis
A0A1C4XM11	A0A1C4XM63	Micromonospora coriariae
A0A1C5CP40	A0A1C5CPH1	Streptomyces sp. Ncost-T10-10d
A0A1C5D1B7	A0A1C5D1A6	Streptomyces sp. Cmuel-A718b
A0A1C5FIC7	A0A1C5FJB4	Streptomyces sp. MnatMP-M17
A0A1C5G7Q8	A0A1C5G8S6	Micromonospora echinofusca
A0A1C5GPW7	A0A1C5GQK8	Micromonospora zamorensis
A0A1C6NPX7	A0A1C6NPH8	Streptomyces sp. AmelKG-D3
A0A1C6UQD4	A0A1C6UQP0	Micromonospora eburnea
A0A1C6VY14	A0A1C6VY60	Micromonospora peucetia
A0A1E5PVW4	A0A1E5Q214	Streptomyces subrutilus
A0A1E7N9W0	A0A1E7NAH0	Kitasatospora aureofaciens
A0A1E7N9W6	A0A1E7NA64	Kitasatospora aureofaciens
A0A1G5GGQ1	A0A1G5GGI7	Streptomyces sp. 136MFCol5.1
A0A1G5JV31	A0A1G5JVA0	Streptomyces sp. 136MFCol5.1
A0A1G6WPA2	A0A1G6WPJ5	Alloactinosynnema iranicum
A0A1G7C1E1	A0A1G7C1R1	Streptomyces jietaisiensis
A0A1G7LZV4	A0A1G7M0C7	Streptomyces jietaisiensis
A0A1G7XUG5	A0A1G7XUG0	Streptomyces jietaisiensis
A0A1G8WML1	A0A1G8WMP2	Nonomuraea maritima
A0A1G9DA01	A0A1G9D9E5	Nonomuraea jiangxiensis
A0A1G9PDZ7	A0A1G9PD87	Streptomyces wuyuanensis
A0A1H0D7U0	A0A1H0D7N6	Streptomyces wuyuanensis
A0A1H0WZZ7	A0A1H0WZZ1	Lentzea jiangxiensis
A0A1H2C4Q2	A0A1H2C3L8	Actinoplanes derwentensis
A0A1H2CWI0	A0A1H2CVZ5	Streptomyces sp. 2114.2
A0A1H4TIP6	A0A1H4TIA0	Streptomyces sp. 2131.1
A0A1H5MF42	A0A1H5MGQ9	Streptomyces sp. Ag109_O5-10

A0A1H5MSX2	A0A1H5MT11	Streptomyces sp. Ag109_O5-10
A0A1H5VHM3	A0A1H5VJ45	Streptomyces yanglinensis
A0A1H5XYE0	A0A1H5XX26	Actinomadura echinospora
A0A1H5ZY41	A0A1H5ZVE5	Actinomadura echinospora
A0A1H6YBE7	A0A1H6Y914	Xiangella phaseoli
A0A1H7G2N2	A0A1H7G2Y5	Streptacidiphilus jiangxiensis
A0A1H9WH15	A0A1H9WGM3	Actinokineospora terrae
A0A1H9WRT3	A0A1H9WS35	Streptomyces sp. yr375
A0A1I0LMG3	A0A1I0LMI5	Nonomuraea wenchangensis
A0A1I2I7E5	A0A1I2I5Q1	Streptomyces alni
A0A1I2JTC6	A0A1I2JW35	Actinoplanes philippinensis
A0A1I3ZHI7	A0A1I3ZIA4	Streptosporangium canum
A0A1I4X566	A0A1I4X4G5	Streptomyces sp. cf124
A0A1I5AVC1	A0A1I5AVB1	Streptomyces sp. cf124
A0A1I6CRS4	A0A1I6CS20	Lentzea waywayandensis
A0A1I6D2T8	A0A1I6D2V8	Lentzea waywayandensis
A0A1I6UEE3	A0A1I6UEC1	Streptomyces harbinensis
A0A1K1VQJ3	A0A1K1VQP5	Streptomyces atratus
A0A1L7GCD1	A0A1L7GQF0	Streptomyces sp. TN58
A0A1L7GJB8	A0A1L7GRF4	Streptomyces sp. TN58
A0A1L9DLD7	A0A1L9DXE1	Streptomyces viridifaciens
A0A1L9DLD8	A0A1L9DLG1	Streptomyces viridifaciens
A0A1M5XAY4	A0A1M5XB19	Streptomyces sp. 3214.6
A0A1M6SYF3	A0A1M6SYI1	Nocardiopsis flavescens
A0A1M6V6Y1	A0A1M6V748	Streptomyces paucisporeus
A0A1N7CYY2	A0A1N7CYZ5	Microbispora rosea
A0A1Q4XR29	A0A1Q4XQY2	Streptomyces sp. CB03911
A0A1Q4XRD0	A0A1Q4XQY2	Streptomyces sp. CB03911
A0A1Q4Y4D4	A0A1Q4Y5E8	Streptomyces sp. CB03578
A0A1Q5BD81	A0A1Q5BE10	Streptomyces sp. MJM1172
A0A1Q5E401	A0A1Q5E343	Streptomyces sp. CB01249
A0A1Q5EUX8	A0A1Q5EUW4	Kitasatospora sp. CB01950
A0A1Q5HGD5	A0A1Q5HGB9	Streptomyces sp. CB01580
A0A1Q5KB04	A0A1Q5K8H5	Streptomyces sp. CB02460
A0A1Q5LG09	A0A1Q5LG54	Streptomyces sp. CB03234
A0A1Q5MNP9	A0A1Q5MP57	Streptomyces sp. CB02488
A0A1Q5N2E5	A0A1Q5N491	Streptomyces sp. CB00455
A0A1Q8UE70	A0A1Q8UE52	Streptomyces sp. MNU77
A0A1Q9LP82	A0A1Q9LPA1	Actinokineospora bangkokensis
A0A1Q9UI73	A0A1Q9UI65	Actinomadura sp. CNU-125
A0A1R3UXA7	A0A1R3UU34	Nocardiopsis sp. JB363
A0A1S1QFV2	A0A1S1QJP0	Frankia sp. Cc1.17
A0A1S1QTS7	A0A1S1QQZ1	Frankia sp. EUN1h
A0A1S1R984	A0A1S1R2X2	Frankia sp. EUN1h
A0A1S1RWC7	A0A1S1RUL9	Frankia sp. BMG5.36
A0A1S2PZI1	A0A1S2PWY7	Streptomyces sp. MUSC 1
A0A1T3NV05	A0A1T3NV01	Embleya scabrispora
A0A1U9P2I3	A0A1U9P9Y3	Streptomyces sp. fd1-xmd
A0A1V0ABT3	A0A1V0ALM0	Nonomuraea sp. ATCC 55076
A0A1V0QZ43	A0A1V0RBQ3	Streptomyces sp. Sge12
A0A1V0R6L6	A0A1V0RCA9	Streptomyces sp. Sge12

A0A1V2IMT1	A0A1V2IMT6	Frankia sp. BMG5.30
A0A1V2KR92	A0A1V2KQT6	Frankia sp. CcI49
A0A1V2QLX0	A0A1V2QLW7	Saccharothrix sp. ALI-22-I
A0A1V2RG86	A0A1V2RG00	Streptomyces sp. MP131-18
A0A1V9KL43	A0A1V9KLA1	Streptomyces sp. M41(2017)
A0A1V9WGR4	A0A1V9WHG6	Streptomyces sp. B9173
A0A1W7CW67	A0A1W7CV74	Streptomyces sp. SCSIO 03032
A0A1X1NKK3	A0A1X1NKM4	Streptomyces sp. CB03238
A0A209CGC9	A0A209CGU5	Streptomyces sp. CS227
A0A209CMP7	A0A209CMS7	Streptomyces sp. CS057
A0A212SLW0	A0A212SLC0	Streptomyces sp. PgraA7
A0A239B847	A0A239B9P7	Actinoplanes regularis
A0A239NIM8	A0A239NHP3	Actinomadura meyerae
A0A239P8P8	A0A239P749	Asanoa hainanensis
A0A249LUQ9	A0A249LUL9	Streptomyces sp. CLI2509
A0A285QR51	A0A285QM97	Streptomyces sp. 1331.2
A0A286EAG3	A0A286EAI9	Streptomyces sp. 1222.2
A0A286ECT3	A0A286ECS4	Streptomyces sp. 1222.2
A0A286EZA4	A0A286EZ49	Streptomyces sp. 1222.2
A0A2A3GYD4	A0A2A3GZ55	Streptomyces sp. Tue6028
A0A2A3I5U1	A0A2A3I3N7	Streptomyces sp. TLI_235
A0A2A4KLS7	A0A2A4KLL5	Streptomyces sp. WZ.A104
A0A2B8ATJ3	A0A2B8B2U6	Streptomyces sp. Ru87
A0A2C9ZLR6	A0A2C9ZLR9	Streptosporangium minutum
A0A2D3U667	A0A2D3UJJ6	Streptomyces peucetius subsp. caesius ATCC 27952
A0A2G5IZM1	A0A2G5J039	Streptomyces sp. HG99
A0A2G6XEV4	A0A2G6XF34	Micromonospora sp. CNZ299
A0A2G7A2P2	A0A2G7A0G6	Streptomyces sp. 1121.2
A0A2G7CIN7	A0A2G7CIZ2	Streptomyces sp. 61
A0A2G7DAJ2	A0A2G7D841	Verrucosispora sp. CNZ293
A0A2G9DPW9	A0A2G9DPJ2	Streptomyces sp. JV178
A0A2H5B440	A0A2H5B445	Kitasatospora sp. MMS16-BH015
A0A2I0SKU9	A0A2I0SKT5	Streptomyces populi
A0A2K8PCN9	A0A2K8PFH7	Streptomyces lavendulae subsp. lavendulae
A0A2L2MIY2	A0A2L2MIX6	Streptomyces dengpaensis
A0A2M9I333	A0A2M9I3R2	Streptomyces sp. TSRI0384-2
A0A2M9K385	A0A2M9K3V0	Streptomyces sp. CB01635
A0A2M9KAY5	A0A2M9KAK8	Streptomyces sp. CB02120-2
A0A2M9KCW3	A0A2M9KDT5	Streptomyces sp. CB02120-2
A0A2M9LGU6	A0A2M9LGW6	Streptomyces sp. CB02613
A0A2N0FHQ9	A0A2N0FHR4	Streptomyces sp. 4121.5
A0A2N0GTZ4	A0A2N0GU84	Streptomyces sp. Ag109_G2-1
A0A2N0IYT9	A0A2N0IYW6	Streptomyces sp. 69
A0A2N0JRS8	A0A2N0JRS9	Kitasatospora sp. OK780
A0A2N3K0G0	A0A2N3K0G5	Streptomyces sp. EAG2
A0A2N3UQP3	A0A2N3UQM9	Streptomyces sp. GP55
A0A2N3VTJ9	A0A2N3VTA9	Streptomyces sp. TLI_146
A0A2N3Y6P3	A0A2N3Y6N8	Saccharopolyspora spinosa
A0A2N3YZW9	A0A2N3YZW5	Micromonospora sp. CNZ309
A0A2N7T251	A0A2N7T260	Verrucosispora sp. ts21

A0A2N9B2G6	A0A2N9B2E9	Streptomyces chartreusis NRRL 3882
A0A2P7PXG1	A0A2P7PXA9	Streptosporangium nondiastaticum
A0A2P7Z906	A0A2P7Z8Y6	Streptomyces sp. 111WW2
A0A2P8BLH9	A0A2P8BLG8	Streptomyces sp. CS149
A0A2P8I3F8	A0A2P8I3H1	Saccharothrix carnea
A0A2P8PWL1	A0A2P8PWM4	Streptomyces sp. A217
A0A2P9EW35	A0A2P9EW49	Streptomyces sp. MA5143a
A0A2P9I985	A0A2P9I9S2	Actinomadura parvosata subsp. kistnae
A0A2R4FSX3	A0A2R4FSZ2	Plantactinospora sp. BB1
A0A2R4JG02	A0A2R4K067	Streptomyces sp. P3
A0A2R4SZB8	A0A2R4TDW9	Streptomyces lunaelactis
A0A2S1SQ83	A0A2S1SQG2	Streptomyces tirandamycinicus
A0A2S1YWM4	A0A2S1YWL3	Streptomyces spongiicola
A0A2S2FUZ4	A0A2S2FUN9	Streptomyces sp. SM17
A0A2S2G322	A0A2S2GHB9	Streptomyces sp. SM18
A0A2S3Y395	A0A2S3Y362	Streptomyces sp. ZL-24
A0A2S4XWX5	A0A2S4XX30	Streptomyces sp. Ru73
A0A2S4YJA9	A0A2S4YJL5	Streptomyces sp. Ru71
A0A2S6PXE9	A0A2S6PXF1	Streptomyces sp. QL37
A0A2S6WLF2	A0A2S6WLA7	Streptomyces sp. MH60
A0A2S6WPG0	A0A2S6WPF7	Streptomyces sp. 46
A0A2S9PN61	A0A2S9PNB9	Streptomyces sp. ST5x
A0A2T0SWN1	A0A2T0SWM3	Umezawaea tangerina
A0A2T7L4S6	A0A2T7L4L8	Streptomyces sp. CS131
A0A2T7L5C6	A0A2T7L5C0	Streptomyces sp. CS014
A0A2T7M489	A0A2T7M3S8	Streptomyces sp. CS090A
A0A2T7MNZ3	A0A2T7MP23	Streptomyces sp. CS147
A0A2T7T7D5	A0A2T7T7K1	Streptomyces scopuliridis RB72
A0A2V1NLR3	A0A2V1NLH9	Streptomyces sp. V2
A0A2V2ATG9	A0A2V2B402	Streptomyces sp. CG 926
A0A2V4NJ29	A0A2V4P5V2	Streptomyces tateyamensis
A0A2W2CFV4	A0A2W2DMC0	Jishengella endophytica
A0A2W2CGD1	A0A2W2DGS8	Micromonospora deserti
A0A2W2CK63	A0A2W2CYC1	Jishengella endophytica
A0A2W4QMB1	A0A2W4NJL9	Actinobacteria bacterium
A0A2W6CS80	A0A2W6CMP0	Pseudonocardiales bacterium
A0A2X2P9G4	A0A2X2LZ37	Streptomyces griseus
A0A2X3L6E8	A0A2X3KTN6	Frankia sp. Ea1.12
A0A2Z3UI41	A0A2Z3UJY5	Streptosporangium sp. 'caverna'
A0A2Z4UYC8	A0A2Z4V9U2	Streptomyces sp. ICC1
A0A2Z5JLA6	A0A2Z5JIE4	Streptomyces atratus
A0A2Z5JQL0	A0A2Z5JQD6	Streptomyces atratus
A0A316FCE1	A0A316FAP2	Actinoplanes xinjiangensis
A0A317D4S2	A0A317D6Z3	Micromonospora sp. 5R2A7
A0A317LK75	A0A317LL65	Nocardiopsis sp. L17-MgMaSL7
A0A317S413	A0A317S3M3	Actinokineospora mzabensis
A0A327TDH6	A0A327TE11	Kitasatospora sp. SolWspMP-SS2h
A0A327V4K6	A0A327VFM8	Streptomyces sp. KhCrAH-43
A0A327ZKA7	A0A327ZL08	Actinoplanes lutulentus
A0A344TWD6	A0A344TWD7	Streptomyces globosus
A0A345T341	A0A345T342	Streptacidiphilus sp. DSM 106435

A0A358SNX0	A0A358SPK1	Actinobacteria bacterium
A0A365H3K6	A0A365H138	Actinomadura sp. LHW63021
A0A365HA33	A0A365HAK1	Actinomadura sp. LHW63021
A0A365ZVQ5	A0A365ZVT7	Streptomyces sp. PT12
A0A370B5U2	A0A370B7F4	Streptomyces corynorhini
A0A370BCA7	A0A370BHZ7	Streptomyces corynorhini
A0A370RH18	A0A370RHA5	Streptomyces sp. HB202
A0A372GAG0	A0A372G9I9	Actinomadura sp. LHW52907
A0A380MR20	A0A380MR53	Streptomyces griseus
A0A384I871	A0A384IHN3	Streptomyces sp. AC1-42W
A0A385DA15	A0A385D9S2	Streptomyces koyangensis
A0A388T029	A0A388T3Z5	Streptomyces spongiicola
A0A397QDY9	A0A397QHI3	Streptomyces sp. 19
A0A397R4V6	A0A397R8E8	Streptomyces sp. 3211.1
A0A399H7K0	A0A399H577	Streptomyces sp. YIM 130001
A0A3A9WFN4	A0A3A9VZM8	Streptomyces sp. AZ1-7
A0A3A9YX76	A0A3A9YZ33	Streptomyces hoynatensis
A0A3A9ZWF6	A0A3A9ZZ57	Micromonospora costi
A0A3D8NL33	A0A3D8NL08	Streptomyces sp. IB2014 011-12
A0A3D9QTI2	A0A3D9QR75	Streptomyces sp. 3212.3
A0A3D9SHU3	A0A3D9SIG7	Actinomadura umbrina
A0A3E0GN80	A0A3E0GL89	Streptomyces sp. 2221.1
A0A3G4VQC1	A0A3G4VVX0	Streptomyces sp. ADI95-16
A0A3L7BU08	A0A3L7BU27	Micromonospora sp. BL4
A0A3L7BWZ6	A0A3L7BWY8	Micromonospora sp. CV4
A0A3M8U363	A0A3M8U433	Streptomyces sp. NEAU-LD23
A0A3N1HFV6	A0A3N1HFV9	Saccharothrix texasensis
A0A3N1LYD5	A0A3N1M2N3	Streptomyces ossamyceticus
A0A3N1SEW3	A0A3N1SDZ1	Streptomyces sp. 840.1
A0A3N1SQ42	A0A3N1SL56	Streptomyces sp. 840.1
A0A3N1T3X2	A0A3N1TCT9	Streptomyces sp. CEV 2-1
A0A3N1U4J6	A0A3N1TUF5	Streptomyces sp. CEV 2-1
A0A3N1UY22	A0A3N1UZY1	Streptomyces sp. 2132.2
A0A3N1YVC4	A0A3N1YYB0	Kitasatospora cineracea
A0A3N4RIC0	A0A3N4RXG5	Kitasatospora niigatensis
A0A3N4SQP3	A0A3N4SCI5	Streptomyces sp. Ag109_O5-1
A0A3N5AL06	A0A3N5BB93	Streptomyces sp. Ag109_G2-6
A0A3N6DE32	A0A3N6FXV8	Streptomyces sp. ADI91-18
A0A3N6F4K2	A0A3N6G610	Streptomyces sp. ADI96-02
A0A3N6FQ75	A0A3N6FLE5	Streptomyces sp. ADI97-07
A0A3N6FVN9	A0A3N6EGY5	Streptomyces sp. ADI96-15
A0A3N6FX82	A0A3N6GYK9	Streptomyces sp. ADI95-17
A0A3N6HTX2	A0A3N6GKF1	Streptomyces sp. ADI98-12
A0A3N6I2F3	A0A3N6GAD3	Streptomyces sp. ADI95-17
A0A3Q8W8A6	A0A3Q8WA02	Streptomyces sp. W1SF4
A0A3R9UNN7	A0A429RNX4	Streptomyces sp. WAC06614
A0A3R9UWE6	A0A429RZ95	Streptomyces sp. WAC05292
A0A3R9XGC0	A0A429T9N4	Streptomyces sp. WAC07149
A0A3R9XP27	A0A429UH43	Streptomyces sp. WAC05374
A0A3S8Y671	A0A3Q8W210	Streptomyces sp. W1SF4
A0A3T1AXX7	A0A3T1AXT9	Actinoplanes sp. OR16

A0A401YSF5	A0A401YSE7	Embleya hyalina
A0A418N138	A0A418N231	Micromonospora radicis
A0A421BBS0	A0A421BBP9	Actinokineospora cianjurensis
A0A421LIK8	A0A421LIK4	Streptomyces sp. LaPpAH-201
A0A423V0D6	A0A423V0C4	Streptomyces globisporus
A0A429F8V5	A0A429F8W7	Actinomadura sp. WAC 06369
A0A429I9S6	A0A429I9T4	Streptomyces sp. WAC 06783
A0A429INB7	A0A429ING0	Streptomyces sp. WAC 06725
A0A429QRZ1	A0A3R9VYX6	Streptomyces sp. WAC07061
A0A429T3K9	A0A3R9XB12	Streptomyces sp. WAC05950
A0A429TAN1	A0A3R9VNS4	Streptomyces sp. WAC07149
A0A429TSQ9	A0A3R9VYA9	Streptomyces sp. WAC04770
A0A432N705	A0A432N6W3	Verrucosispora sp. FIM060022
A0A495QKT5	A0A495QL66	Actinomadura pelletieri DSM 43383
A0A495R149	A0A495R032	Actinomadura pelletieri DSM 43383
A0A495TBA2	A0A495TAE3	Streptomyces sp. 1114.5
A0A495W527	A0A495W6M9	Saccharothrix australiensis
A0A495XLA8	A0A495XKM0	Saccharothrix variisporea
A0A498B7J2	A0A498B7I9	Streptomyces sp. 57
A0A4D4J478	A0A4D4J7P2	Gandjariella thermophila
A0A4D4MQX0	A0A4D4MQ65	Streptomyces avermitilis
A0A4P6TZ93	A0A4P6U2L8	Streptomyces seoulensis
A0A4Q6VCA6	A0A4Q6VAZ3	Streptomyces sp. SCA2-2
A0A4Q7Z2M9	A0A4Q7Z4B7	Streptomyces sp. BK022
A0A4Q7ZMV2	A0A4Q7ZMV6	Krasilnikovia cinnamomea
A0A4R0GS97	A0A4R0GXB3	Micromonospora zingiberis
A0A4R1CV15	A0A4V2P0U2	Frankia sp. BMG5.11
A0A4R2AZ35	A0A4R2AYK7	Micromonospora sp. CNZ303
A0A4R2J4A4	A0A4V2S5U4	Actinocrispum wychmicini
A0A4R2QP39	A0A4R2QWF3	Streptomyces sp. BK438
A0A4R3BLI4	A0A4R3BPX5	Streptomyces sp. BK329
A0A4R3CUB3	A0A4R3CTY5	Streptomyces sp. BK038
A0A4R3D3G9	A0A4V2U1S7	Streptomyces sp. BK308
A0A4R3DA40	A0A4R3DC57	Streptomyces sp. BK308
A0A4R3ERL0	A0A4V6NWQ2	Streptomyces sp. BK674
A0A4R3IQ37	A0A4R3IL25	Streptomyces sp. BK335
A0A4R5C851	A0A4R5CAU4	Actinomadura sp. H3C3
A0A4R5FID0	A0A4R5FIL0	Nonomuraea sp. 6K102
A0A4R6VA88	A0A4R6V497	Actinorugispora endophytica
A0A4R7JEF4	A0A4R7JBB6	Streptomyces sp. BK447
A0A4R8HAZ4	A0A4R8HGB2	Streptomyces sp. 25
A0A4V1B1B4	A0A4P7DFY5	Streptomyces sp. S501
A0A4V1VMT8	A0A4Q4DFM2	Streptomyces sp. L-9-10
A0A4V2UM06	A0A4R3IWV4	Streptomyces sp. BK335
A0A4V2XJX9	A0A4R4NAH7	Nonomuraea sp. KC201
A0A4V3ELN6	A0A4R7IS56	Streptomyces sp. BK161
A0A4V6Q5J2	A0A4R7SBU6	Streptomyces sp. KS 21
A0A4Y8NTS5	A0A4Y8NTZ5	Streptomyces sp. ICN441
A0A4Z1DGC7	A0A4Z1DG56	Streptomyces bauhiniae
A0A4Z1DQI7	A0A4Z1DRE3	Streptomyces griseoluteus
A0A504DIH5	A0A504DH74	Mesorhizobium sp. B2-3-3

A0A505DEP4	A0A505DJQ4	Streptomyces sp. NEAU-SSA 1
A0A540Q425	A0A540Q472	Streptomyces ipomoeae
A0A540Q7K4	A0A540Q7Z5	Streptomyces ipomoeae
A0A540Q9U8	A0A540Q9E8	Streptomyces ipomoeae
A0A540QPN3	A0A540NYL6	Streptomyces ipomoeae
A0A540W473	A0A540W471	Kitasatospora sp. MMS16-CNU292
A0A542EYT7	A0A542EYT6	Micromonospora sp. A202
A0A542HUG6	A0A542HU89	Streptomyces sp. SLBN-115
A0A542Q0K0	A0A542Q0N6	Streptomyces sp. SLBN-118
A0A543J3Y2	A0A543J3Y7	Thermopolyspora flexuosa
A0A543JMS0	A0A543JMT3	Saccharothrix saharensis
A0A552R3W3	A0A552R3U5	Streptomyces sp. 130
A0A560A002	A0A560A008	Micromonospora sp. CNZ322
A0A561ETU5	A0A561ETV0	Kitasatospora atroaurantiaca
A0A561RJY9	A0A561RJY3	Streptomyces argenteolus
A0A561UGB9	A0A561UGB0	Kitasatospora viridis
A0A561V213	A0A561V244	Streptomyces brevispora
A0A561VF89	A0A561VFB1	Micromonospora taraxaci
A0A5B8E034	A0A5B8DYW9	Streptomyces albidoflavus
A0A5C4QNY8	A0A5C4QN11	Micromonospora orduensis
A0A5C4W413	A0A5C4W1S7	Nonomuraea phyllanthi
A0A5C6IDZ1	A0A5C6IHR2	Streptomyces albidoflavus
A8M4S4	A8M4S3	Salinispora arenicola (strain CNS-205)
B5HLH5	D6XBR5	Streptomyces sviceus ATCC 29083
B5HUD6	B5HUD5	Streptomyces sviceus ATCC 29083
C7PXA6	C7PXA7	Catenulispora acidiphila (strain DSM 44928 / NRRL B-24433 / NBRC 102108 / JCM 14897)
C9YT11	C9YT10	Streptomyces scabiei (strain 87.22)
C9Z6K5	C9Z6K1	Streptomyces scabiei (strain 87.22)
C9ZC34	C9ZC33	Streptomyces scabiei (strain 87.22)
C9ZCF5	C9ZCF4	Streptomyces scabiei (strain 87.22)
D2B797	D2B794	Streptosporangium roseum (strain ATCC 12428 / DSM 43021 / JCM 3005 / NI 9100)
D3D356	D3D355	Frankia sp. EUN1f
D3D359	D3D355	Frankia sp. EUN1f
D6B6N6	D6B6N7	Streptomyces albidoflavus
D6EUL4	D6EUL3	Streptomyces lividans TK24
D9VPL0	D9VPL1	Streptomyces sp. C
D9VYP9	D9VYQ0	Streptomyces sp. C
D9WR65	D9WR66	Streptomyces himastatinicus ATCC 53653
E3JAZ0	E3JAY9	Frankia inefficax (strain DSM 45817 / CECT 9037 / EuI1c)
E4NFH4	E4NFH5	Kitasatospora setae (strain ATCC 33774 / DSM 43861 / JCM 3304 / KCC A-0304 / NBRC 14216 / KM-6054)
E8W5K9	E8W5L0	Streptomyces pratensis (strain ATCC 33331 / IAF-45CD)
F3NAU0	F3NAU3	Streptomyces griseoaurantiacus M045
F3ND60	F3ND61	Streptomyces griseoaurantiacus M045
F3NGR8	F3NGR7	Streptomyces griseoaurantiacus M045
F3Z709	F3Z708	Streptomyces sp. Tu6071

F4F3S7	F4F3S8	Verrucosispora maris (strain AB-18-032)
F8B685	F8B684	Frankia symbiont subsp. Datisca glomerata
G0Q517	G0Q518	Streptomyces sp. ACT-1
I0H3J3	I0H3J2	Actinoplanes missouriensis (strain ATCC 14538 / DSM 43046 / CBS 188.64 / JCM 3121 / NCIMB 12654 / NBRC 102363 / 431)
I0L5F6	I0L5F7	Micromonospora lupini str. Lupac 08
J7LDH3	J7LJ81	Nocardiopsis alba (strain ATCC BAA-2165 / BE74)
K0K089	K0K5U7	Saccharothrix espanaensis (strain ATCC 51144 / DSM 44229 / JCM 9112 / NBRC 15066 / NRRL 15764)
L1KQP3	L1KQE4	Streptomyces ipomoeae 91-03
L1L497	L1L3D8	Streptomyces ipomoeae 91-03
L7ESL4	L7ETG5	Streptomyces turgidiscabies Car8
L7FBZ3	L7FD96	Streptomyces turgidiscabies Car8
L8EWX8	L8F0S4	Streptomyces rimosus subsp. rimosus (strain ATCC 10970 / DSM 40260 / JCM 4667 / NRRL 2234)
M3D8F8	M3ETS5	Streptomyces bottropensis ATCC 25435
M3ESS4	M3D7E8	Streptomyces bottropensis ATCC 25435
M3EWW5	M3FND2	Streptomyces bottropensis ATCC 25435
Q82BI9	Q82BJ0	Streptomyces avermitilis (strain ATCC 31267 / DSM 46492 / JCM 5070 / NBRC 14893 / NCIMB 12804 / NRRL 8165 / MA-4680)
Q9F3J3	Q9F3J2	Streptomyces coelicolor (strain ATCC BAA-471 / A3(2) / M145)
S2XSG9	S2YU48	Streptomyces sp. HGB0020
V4IV16	V4KJC0	Streptomyces sp. PVA 94-07
W7IT42	W7IFD2	Actinokineospora spheciospongiae
W9FQ90	W9FMS1	Streptomyces filamentosus NRRL 11379

In one embodiment, the rSAM enzyme or enzymatically active fragment has two Cys-rich domains that are critical or essential for activity. The two Cys-rich domains may include the rSAM binding domain in the N-terminus (CXXXCXXC) and the SPASM domain in the C-terminus (CXXXCXXXXXC) or CXXCXXXXXC, where X may be any amino acid).

The term "domain", as used herein, refers to a part of a molecule or structure that shares common physicochemical features, such as, but not limited to, hydrophobic, polar, globular and helical domains or properties such as ligand-binding, membrane fusion, signal transduction, cell penetration and the like. Often, a domain has a folded protein structure which has the ability to retain its tertiary structure independently of the rest of the protein. Generally, domains are responsible for discrete functional properties of proteins, and in many cases may be added, removed or transferred to other proteins without loss of function of the remainder of the protein and/or of the

domain. Domains may be co-extensive with regions or portions thereof; domains may also include distinct, non-contiguous regions of a molecule.

The rSAM enzyme may be a recombinant enzyme or is isolated from bacteria.

The term "recombinant" when used with reference to, e.g., polypeptide, enzyme, nucleic acid or cell refers to a material, or a material corresponding to the natural or native form of the material, that has been modified in a manner that would not otherwise exist in nature, or is identical thereto but produced or derived from synthetic materials and/or by manipulation using recombinant techniques. Non-limiting examples include, among others, recombinant cells expressing genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise expressed at a different level.

In some embodiments, the nucleic acid sequence which encodes a rSAM/SPASM maturase comprises Xye, Grr or Fxs. In other embodiments, the nucleic acid sequence comprises Xye.

In one embodiment, the maturase is an enzyme from the XYE maturase system. The enzyme may be a XyeB SPASM protein (e.g. xncB, ykcB or etcB) or an enzymatically active fragment of the enzyme. The polypeptide may be a polypeptide having at least 80% identity to a XyeA precursor peptide (e.g. xncA, ykcA and etcA), including an XyeA precursor peptide that is listed in Table 4. In one embodiment, the polypeptide comprises $WIX_4AFX_5NWX_6X_7$ (SEQ ID NO: 71), wherein X_4 is N or K, wherein X_5 is G or A, wherein X_6 is E, S or T and wherein X_7 is R or K. The polypeptide may comprise WINAFGNWER (SEQ ID NO: 72), WIKAFGNWSR (SEQ ID NO: 73) or WINAFANWTK (SEQ ID NO: 74), WINAFGNWERAFH (SEQ ID NO: 75), AGWIKAFGNWSRSF (SEQ ID NO: 76) or WINAFANWTKRI (SEQ ID NO: 77).

In one embodiment, the enzyme is an enzyme from the GRR maturase system. The enzyme may be an GrrM SPASM protein (e.g. oscB, lscB or gscB) or an enzymatically active fragment of the enzyme. The enzyme may, for example, act on a peptide having at least 80% identity to an GrrA precursor peptide (e.g. oscA, lscA and gscA), including a GrrA precursor peptide that is listed in Table 5. The polypeptide may comprise

(a) GAWGNGGGRGGWINRGGGGSWGNGGSWRNGGGWRNGWGDGGRFINSR (SEQ ID NO: 78);
(b) GGGFTQGGRRGVATGPRGGNFYNAHPNYGRVGGPVGVGRGAAWADGGGFYNGTYQD GGSFVNGSDGGAAFKNGTYGAGGFVNGSQGGAGFRNW (SEQ ID NO: 79); or
(c) GFANGGGGFANRVGPGGFLNDNGGGGFLNNRGWGDGGGGFLNRR (SEQ ID NO: 80).

In one embodiment, the enzyme is an enzyme from the FXS maturase system. The enzyme may be an FxsB SPASM protein (e.g. mscB) or an enzymatically active fragment of the enzyme. The enzyme may, for example, act on a peptide having at least 80% identity to an FxsA precursor peptide (e.g. mscA), including a FxsA precursor peptide that is listed in Table 6. The polypeptide may comprise IPAAKFSSFI (SEQ ID NO: 81).

The terms "Percentage of sequence identity" and "percentage identity" are used interchangeably herein to refer to comparisons among polynucleotides and polypeptides, and are determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage may be calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Alternatively, the percentage may be calculated by determining the number of positions at which either the identical nucleic acid base or amino acid residue occurs in both sequences or a nucleic acid base or amino acid residue is aligned with a gap to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Those of skill in the art appreciate that there are many established algorithms available to align two sequences. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith and Waterman, 1981, *Adv. Appl. Math.* 2:482, by the homology alignment algorithm of Needleman and Wunsch, 1970, *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson and Lipman, 1988, *Proc. Natl. Acad. Sci. USA* 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT,

FASTA, and TFASTA in the GCG Wisconsin Software Package), or by visual inspection (see generally, *Current Protocols in Molecular Biology*, F. M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1995 Supplement) (Ausubel)). Examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., 1990, *J. Mol. Biol.* 215: 403-410 and Altschul et al., 1977, *Nucleic Acids Res.* 3389-3402, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information website. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as, the neighborhood word score threshold (Altschul et al, supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=−4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff, 1989, *Proc Natl Acad Sci USA* 89:10915). Exemplary determination of sequence alignment and % sequence identity can employ the BESTFIT or GAP programs in the GCG Wisconsin Software package (Accelrys, Madison Wis.), using default parameters provided.

The term "nucleic acid" includes a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogues of natural nucleotides that hybridize to nucleic acids in a manner

similar to naturally occurring nucleotides. The terms "nucleic acid", "nucleic acid molecule", "nucleic acid sequence" and polynucleotide etc. are used interchangeably herein unless the context indicates otherwise.

As used herein, the terms "encode", "encoding" and the like refer to the capacity of a nucleic acid to provide for another nucleic acid or a polypeptide. For example, a nucleic acid sequence is said to "encode" a polypeptide if it can be transcribed and/or translated to produce the polypeptide or if it can be processed into a form that can be transcribed and/or translated to produce the polypeptide. Such a nucleic acid sequence may include a coding sequence or both a coding sequence and a non-coding sequence. Thus, the terms "encode", "encoding" and the like include a RNA product resulting from transcription of a DNA molecule, a protein resulting from translation of a RNA molecule, a protein resulting from transcription of a DNA molecule to form a RNA product and the subsequent translation of the RNA product, or a protein resulting from transcription of a DNA molecule to provide a RNA product, processing of the RNA product to provide a processed RNA product (e.g., mRNA) and the subsequent translation of the processed RNA product.

The term "construct" refers to a recombinant genetic molecule including one or more isolated nucleic acid sequences from different sources. Thus, constructs are chimeric molecules in which two or more nucleic acid sequences of different origin are assembled into a single nucleic acid molecule and include any construct that contains (1) nucleic acid sequences, including regulatory and coding sequences that are not found together in nature (i.e., at least one of the nucleotide sequences is heterologous with respect to at least one of its other nucleotide sequences), or (2) sequences encoding parts of functional RNA molecules or proteins not naturally adjoined, or (3) parts of promoters that are not naturally adjoined. Representative constructs include any recombinant nucleic acid molecule such as a plasmid, cosmid, virus, autonomously replicating polynucleotide molecule, phage, or linear or circular single stranded or double stranded DNA or RNA nucleic acid molecule, derived from any source, capable of genomic integration or autonomous replication, comprising a nucleic acid molecule where one or more nucleic acid molecules have been operably linked. Constructs of the present invention will generally include the necessary elements to direct expression of a nucleic acid sequence of interest that is also contained in the construct, such as, for example, a target nucleic acid sequence or a modulator nucleic acid sequence. Such elements

may include control elements such as a promoter that is operably linked to (so as to direct transcription of) the nucleic acid sequence of interest, and often includes a polyadenylation sequence as well. Within certain embodiments of the invention, the construct may be contained within a vector. In addition to the components of the construct, the vector may include, for example, one or more selectable markers, one or more origins of replication, such as prokaryotic and eukaryotic origins, at least one multiple cloning site, and/or elements to facilitate stable integration of the construct into the genome of a host cell. Two or more constructs can be contained within a single nucleic acid molecule, such as a single vector, or can be containing within two or more separate nucleic acid molecules, such as two or more separate vectors. An "expression construct" generally includes at least a control sequence operably linked to a nucleotide sequence of interest. In this manner, for example, promoters in operable connection with the nucleotide sequences to be expressed are provided in expression constructs for expression in an organism or part thereof including a host cell. For the practice of the present invention, conventional compositions and methods for preparing and using constructs and host cells are well known to one skilled in the art, see for example, Molecular Cloning: A Laboratory Manual, 3rd edition Volumes 1, 2, and 3. J. F. Sambrook, D. W. Russell, and N. Irwin, Cold Spring Harbor Laboratory Press, 2000.

By "control element" or "control sequence" is meant nucleic acid sequences (e.g., DNA) necessary for expression of an operably linked coding sequence in a particular host cell. The control sequences that are suitable for prokaryotic cells for example, include a promoter, and optionally a cis-acting sequence such as an operator sequence and a ribosome binding site. Control sequences that are suitable for eukaryotic cells include transcriptional control sequences such as promoters, polyadenylation signals, transcriptional enhancers, translational control sequences such as translational enhancers and internal ribosome binding sites (IRES), nucleic acid sequences that modulate mRNA stability, as well as targeting sequences that target a product encoded by a transcribed polynucleotide to an intracellular compartment within a cell or to the extracellular environment.

In some embodiments, the precursor polypeptide and the rSAM enzyme are selected from the following Table 7.

Table 7. Combination of precursor polypeptide sequence and rSAM sequence.

Product name	Core sequence[a]	MW[b]	Genus	XyeCDE[c]	Precursor ID[d]	Precursor sequence[d]	rSAM ID[d]	rSAM sequence[d]
	WVNAFANWSKAL	1400.56	*Xenorhabdus*	CDE	WP_072032494.1	MSKLQREIA ENKAQVTN SDKNKTQS KELVDNLLD TVSGGWVN AFANWSKA L (SEQ ID 82)	WP_187650499.1	MAIVKNEKIKHIEIILKISERCNINCTY CYVFNMGNTLAADSTPIISLDNVAALR GFFERSVIENEIEVIQVDFHGGEPLMM KKERFNRMCEILREGNYGSSRLVLAL QTNGILIDDEWIALFEKHQVHASISID GPKHINDRHRLDQKGKSTYEGTVKGL RMLQNAWAQGRIPVEPGILSVANAKA NGEEIYHHFSKELKCQRFDFLIPDDQH TDGIDAEGIGRFLNEALDAWFADGQP NIFVRIFNTYLGTMLNNQFSRVLGISA NVESAYAFTVTSDGLLRIDDTLRSTSD KIFNSIGHVSKLTLASVLESSNVREYL SLSDELPDACCGCIWSKVCHGGRLVN RFSQTNRFHNKTVFCPSMRLFLSRAA SHLIAAGISEETIIENIQK (SEQ ID 138)
	WVNAFGNWSKSL	1402.53	*Xenorhabdus*	CDE	WP_099120413.1	MSKLQREIA ENKSQIVN SDKNKTQR KELVDGLLD TVSGGWVN AFGNWSKS L (SEQ ID 83)	WP_099120414.1	MAIIKNEKIKHLEIILKVSERCNINCTY CYVFNMGNTLAADSAPIISLDNIAALR GFFERSVIENHIEVIQVDFHGGEPLMM KKERFNQMCEILREGNYGNSQLVLAL QTNGILIDDEWIALFEKHQVHASISID GPKHINDRHRLDRKGKSTYEGTVNGL RMLQNAWAQGRIPAEPGILSVANANA NGGEIYHHFSKELKCQRFDFLIPDDQ HADSTDAEGIGRFLNEALDAWFADG QPNIFVRIFNTYLGTMLNSQFHRIIGIS ANVESVYAFTVTSDGLLRIDDTLRSTS DKIFNPIGHVRELTLSSVLESTNAKEY SSLNSELPEDCNDCIWSKICHGGRLV NRFSPTNRFHNKTVFCPSMRVFLSRA ASHLIEAGVSEETIIKNIQQ (SEQ ID 139)

	WVNAFANWSKSF	1450.58	*Xenorhabdus*	CDE	WP_193850059.1	MSKLQREIVENKTQVTNSDKNKAQRKELVDSLLDTVSGGWVNAFANWSKSF (SEQ ID 84)	WP_193850057.1	MAIVKDGKVKHLEVILKISERCNINCTYCYVFNMGNTLAADSAPVISLDTVASLREFFERSVVENEIEVIQVDFHGGEPLMMKKERFNRMCEILREGNYGRSRLVLALQTNGILIDNEWISIFEKHQIHVSVSIDGPKHINDRYRLDRKGKSTYEGTVNGLRMLQNAWTQGRLSGEPGILSVANAKANGEEIYRHFTKELKCQRFDFLIPDDQHADSIDVEGIGRFLNEALDAWFADGQPKIFIRIFNTYLGTMLNNQFSRVLGMSANVESAYAFTVTADGQLRVDDTLRSTSDQIFSAIGHVSELTLARVLESPNVKEYLSLSSELPDACCGCVWSKICHGGRLVNRFSRANRFHNKTVFCLSMRLFLSRAASHLIAAGVSEETIIENIQK (SEQ ID 140)
	WVNAFARWGKSF	1462.63	*Erwinia;Kosakonia;Pantoea*	CDE	WP_133622747.1	MSKLSKEIAKNQAEVITSKDRNEEKKALAQSMLDSISGGWVNAFARWGKSF (SEQ ID 85)	WP_133622746.1	MKNWSQNDLKKIKHLEIILKVSERCNINCSYCYMYNLGNNISIKSKPVIPFSVVKDLRNFFEQATKEHEIETIQVDFHGGEPLMMGKERFEVACDELAKGHYKNTKLNMACQTNATLIDDEWIEVFSKYNISVGISIDGPKHINDKHRLDKKGRSTYDKKVNGLKMLQKAWQEGKLADEPGILCVANQSVNGAEIYRHFVDDLKSKKFDFLIPDESHDTCSNPDGLSKFYCDAMDEFFSDANKNVYVRYFHTHMQSMLSQEFRPVMGISKSNDDILAFTVCSNGDIYIDDTLRATNDSIFTPIGNIKNLTLSDALSSWQMKKYILIKKTLPENCTDCVWKKICGGGRHIQRYSKDDDFNRETVFCPSIRKIMSRAASHLISSGIPEEKIMMNLEII (SEQ ID 141)
	WVNAFARWGRAF	1474.65	*Yersinia*	DEC	WP_212585760.1	MSRLKKEIIATKTVVNVSEAKRNQPQRLAEDVLEQVAGGWVNAFARWGRAF (SEQ ID 86)	WP_212585759.1	MVNISSKKNIQHLEVILKISERCNINCDYCYVFNKGNSISDNSPARISSENINQLVYFLQRACLEYDIATLQIDFHGGEPLLMKKENFARMCDQLVTADYGGSNINLALQTNGTLVDDEWISLFEKYSVNASVSIDGPKHINDRHRLDTKGRSTYEGTVRGLRMLQKAYQQGRIPSEPGILCVADASVDGAEIYRHFVDELGVYSFDFLIPDDCYKDTHVDAIGMGRFLNEALDEWVKDDNPKVFVRLFQTHIASLLGQMNSGVLGHNPNVTGIYALTVSSDGLVRVDDTLRSTSDSMFNPIGHMSEISLLDVFDSQQFREYSLIGQSLPTECTGCIWENICAGGRIVNRFSPEDRFNRKSTYCYSMRSFLSRASAHLLNMGIKEERIMAAISQ (SEQ ID 142)

	WVNAFVNWPKSF	1488.67	*Yersinia*	DEC	WP_072082693.1	MSRLQKEINETKTVINICNTKKSQPQHLADSILDKIAGGWVNAFVNWPKSF (SEQ ID 87)	WP_050115763.1	MVNQLNIQSIQHLEIILKISERCNINCDYCYVFNKGNPAANNSPARLSDRNINDLAEFLHTACREYKIGTLQIDFHGGEPLLMKKENFAKMCERLLTGRYSKTNIRFALQTNGTLIDEEWISLFEKYSVNASISIDGPKHINDRHRLDTKGRSTYEATVRGLRILQHAHKQGRIPSAPGVLCVANAQANGAEIYRHFVDELKVYGFDFLVPDDCYHDTNIDPVGISRFLNEALDEWFKDSNPNIFVRLFQTHLAHLLGTKHQGILGHSPSATGAYAFTVGSDGFIRVDDTLRATSDRIFNPIGHVSEISLTDALNSPQFQEYASVGQALPHECNGCIWENVCAGGRIMNRFSPETRFDRKSVYCYSMRSFLSRAAAHLLNMGIKEERIMTAIGR (SEQ ID 143)
	WINAFARWGRAF	1488.67	*Yersinia*	DEC	WP_071984901.1	MSSLKKEIMATKTVVNVSEAKRNHPQRLAEDVLEQIAGGWINAFARWGRAF (SEQ ID 88)	WP_054871968.1	MVNISSKKSIQHLEIILKISERCNINCDYCYVFNKGNSIADNSPARISNKNIEQLVYFLQRACLEYDIATLQIDFHGGEPLLMKKENFASMCDQLTTADYGSSNISLALQTNGTLIDDEWISLFEQYLVYVSISIDGPKHINDRHRLDTKGRSTYEGTVRGLRMLQNAYKQGRLQAEPGILCVANPQANGAEIYRHFVDDLGVYGFDILIPDDAYNDTYADPVSMGRFLNEALDEWMKDDNPKIFVRLFQTHIATLLGAKKVGVLGHTPEVTGTYACTVGSDGLIRVDDTLRSTSDRIFNAIGHVSEINLSDVINSPQFQEYVSIGKSLPTECTGCIWENVCAGGRIMNRFSPEERFNRKSVYCYSMRSFLSRASAHLLNMGIKEERIMAAISQ (SEQ ID 144)
Xenorceptide A	WVNAFARWSKSF	1492.66	*Serratia*	CDE	WP_071845309.1	MSKLAKEINMNKAAVTVAADKKDARKALAQSMLDSVSGGWVNAFARWSKSF (SEQ ID 89)	WP_047728930.1	MTNKKKIKHLEIILKVSERCNINCTYCYVFNLGNDLAINSKPIISHKIIEDLRGFFERACQEYEIETVQVDFHGGEPLMMGKERFDNACKELISGDYNGARLNLACQTNAILIDNEWIDIFSKYNISVGISIDGPKHINDRHRLDRKGRSTYEGTVKGLEMLQVAWKAGRLIDEPGILCVANPSVKGAEIYRHFVDVLKCKKFDFLIPDESHDTCTDPDGLADFYCSALDEFFLDADKEVYVRYFHTHIQSMLSSEFNPVMGVSKAGNDTLAFTVSSDGELYVDDTLRATNDPIFTPIGNIQHLILSDTLASWQMTKYMAVNSQLPTVCGDCVWQKVCGGGRHIQRYSTADDFNRETVFCPSVRKIMSRAASHLIESGVAEDIIMKNLEVNS (SEQ ID 145)

	WVNAFVNWTKSF	1492.66	*Yersinia*	DEC	WP_219657009.1	MSRLQKEINETKTVINICNTKKSQPQHLADSILDKIAGGWVNAFVNWTKSF (SEQ ID 90)	WP_219657008.1	MVNQLNMQSIQHLEIILKISERCNINCDYCYVFNKGNPAANNSPARLSDKNINALAELLHTACREYKIGTLQIDFHGGEPLLMKKENFAKMCERLPAGKYSKTNVRFALQTNGTLIDEEWISLFEKYSVNASISIDGPKHINGRHRLDTKGRSTYEATVRGLRILQHAHKQGRIPSAPGVLCVANAQANGAEIYRHFVDDTLRATSDRIFNPIGHVSEISLTDALNSPQFQEYTSIGQSLPHECNGCIWENVCAGGRIMNRFSPETRFDRKSVYCYSMRSFLSRTAAHLLNMGIKEERIMAAIQA (SEQ ID 146)
	WVNVFARWDKAI	1498.71	*Xenorhabdus*	CDE	WP_071839243.1	MRKLQREIALNNAKVINNSEKKQERKVLVENLMDSVSGGWVNVFARWDKAI (SEQ ID 91)	WP_046338175.1	MITKKKIKHLEIILKVSERCNINCTYCYVFNLGNEISINSKPIISHDIIKVLRAFFEQASQEYDIETIQVDFHGGEPLMMGKEKFENACNEFISGSYNKTKFNLACQTNAILIDNEWIDIFSKYNVSVGISIDGPKHINDKHRLDRKGRSTYEGTVRGLVMLQEAWSAGRLIDQPGILCVANPSVKGAEIYRHFVDVLKCKKFDFLIPDESHDTCTNPDGLSDFYCSAIDEFFSDADQDVYVRYFLTHMQSMLSSEFSPVMGLSKSGSDTIALTVSSEGDIYVDDTLRSTNDPIFTPIGNVLNLTLSETIASWQMQKYMTVNNQLPTACTDCIWKKVCGGGRHIQRYSKADDFKRESVFCPSIRKIMSRAASHLIESGISEDIIMKNLGIKSVNRFSQANRFNNKTVFCSSMRIFLSRGASHLMATGIDERTIMANIQG (SEQ ID 147)
	WVNAFANWTKRI	1499.69	*Erwinia*	CDE	WP_082262368.1	MSKLQREITSNKAQLVNADARKMQRKVLVDSLLDTVSGGWVNAFANWTKRI (SEQ ID 92)	WP_168401143.1	MRLIKGEKIKHLEIIFQVSERCNISCTYCYVFNMGNTLAADSHPTISLNNVIALRGFFERSTAENEIEVIQVDFHGGEPLMMKKDRFDQMCHILLQGDYGNSRIELALQTHGILVDEEWITLFEKYKVHASISVDGPKHINDRHRLDRKGKSTYEGTINGLRLLQNAWQQGRLPAEPGILSVANAKANGADIYHHFVDVLKCQRFDFLIPDDHHDDITDSEGIGRFLNEALDAWFADGRAELFVRIFNTYLGTLLDKQFSRVLGMSANVESAYAFTVTADGLLRIDDTLRSTSDEIFNPVGHVRDLSLAGVLKNTAVEEYLSLSNTLPEGCKDCVWNNVCHGGRLVNRFSQANRFNNKTVFCSSMRIFLSRGASHLMATGIDERTIMANIQG (SEQ ID 148)

	WVNAFLRWGKSF	1504.71	*Yersinia*	DEC	WP_071840519.1	MSRLKKEITATKTVINVSEVKKNQPQRLAEDVLEQISGGWVNAFLRWGKSF (SEQ ID 93)	WP_145595300.1	MVNISSEKRIKHLEIILKISERCNINCDYCYVYNKGNTIADNSPARISNKNILQLVDFLQRACREYSIGTLQIDLHGGEPLLMKKENFASMCELLMMADYCGSNINLALQTNGTLVDDEWISLFEKYSIHVSISIDGPKHINDRHRLDTKGRSTYEGTVRGLRRLQHAHQQGRLRAAPGILCVANPQASGTEIYRHFVDDLGVYGFDLLIPDDAYSDDHVDPISMGRFLNEALDEWVKDDNPKIFVRLFQTHIATLLGAKVGVLGHTPEVTGAYACTVGSDGFIRVDDTLRATSDRIFDPIGHVSDISLSEVLDSPQFQEYTLIGQSLPTECENCIWAKVCAGGRIMNRFSPEDRFNRKSVYCYSMRSFLSRASAHLLNMGIKEERIMAAISQ (SEQ ID 149)
Xenorceptide A3	WINAFANWTKRI	1513.72	*Erwinia*	CDE	WP_017801003.1	MSKLQHEIASNKARLNNADDKKAQRKILVDSLLDTVSGGWINAFANWTKRI (SEQ ID 94)	WP_017801004.1	MTQLKGEKIKHLEIILKISERCNINCTYCYVFNMGNTLATDSTPVISLDNVYALRGFFERSAAENDIEVIQVDFHGGEPLMMKKDRFDRMCQILLQGNYRSSKFELALQTNGILIDDEWIALFEKHQVHASISVDGPKHINDRHRLDRKGKSTYEGTITGLRLLQNAWQQGRLPGEPGILSVANANANGAEIYRHFADTLQCQRFDFLIPDDHHDDSPDGEGVGRFLNEALDAWFADGRPEIFIRIFNTYLGTMLNSQFNRVLGMSANVESAYAFTVTADGMLRIDDTLRSTSDEIFNAVGHVSELSLARVLETSCVKEYLALSSNLPTVCAECVWNNICHGGRLVNRFSRTNRFNNKTVFCKSMRLFLSRAASHLMASGVDEKEIMKNIQK (SEQ ID 150)
	WVNAFAKWTKRI	1513.76	*Photorhabdus*	DEC	WP_172908095.1	MSSLKREIAETKTEIKGTKVKNNQPQPLTEDLLDQISGGWVNAFAKWTKRI (SEQ ID 95)	WP_172908148.1	MVNSLVKKKIQHLEVILKISERCNINCDYCYVFNKGNSAANDSPARISHANIDYLVDFFQRGSQEYDIDTLQIDFHGGEPLMMKKQQFASMCDRLASGNYHGSNIKFALQTNGILIDDEWISLFEKYSVSVSVSIDGPKHINDRHRLDRKGRSTYEGTVRGLRKLQEAYQAGRLPSDPGILCVANAKASGAEIYRHFVDNLGVYGFDFLVPDDCYTDALVDPVGVGRFLNEALDEWVNDNNPKIFVRLFNTHIASLLGAENAGFLGHNPSVAGIYAFTIGSDGSVRIDDTLRSTSDRIFDIIGHISEISLSEVLNSPQFQEYVSIGQSLPTECEDCIWAKICAGGRIVNRFSHEERFKRKSVYCYSMRSLLGRVSAHLLNMGIEEDRIMKAISR (SEQ ID 151)

	WVNFFAKFTKSF	1515.73	*Salmonella*	CDE	WP_153789637.1	MSKLMKEIEKQNAKVTVNNKDKVASRKELTDAVLDSITGGWVNFFAKFTKSF (SEQ ID 96)	WP_153789560.1	MPPFKGGLLMNKEKFNFLEIVLKVSERCNINCDYCYMYNCGNELSINSRPLINDETVYNLKKLLENAASEFEIGTIQVDFHGGEPLMLGKRKFSEACDILLSGNYHNSYFILSCQTNGTLIDEEWVDIFYKYNVRIGISIDGPKHINDKHRLDHKGKSTYERTVKGIKMINSAWKKGIMTNEPSILCVINPKVSGKEIYRHFVDDLECKSFDLLIPDENHDTCENTKAVGLYLNEAVDEFFNDSNKEIEVRIIATHMKSLMLKEFTPVIGISKGDINSAVFVITSEGDIYIDDALRVTNDILFSPIGNLRNVKFKNLLESWQLKQYMNINNTLPSSCYDCIWKNSCFGGRALNRFSKVNRFDNKTVFCDSMRIFLSRLTSHIIESGVDIKLIEENLGVNEL (SEQ ID 152)
	WVNAFLNWSRSF	1520.67	*Yersinia*	DEC	WP_074006888.1	MSRLKKEITETKTAIGTNKAKKNQPQHLADDLLDQIAGGWVNAFLNWSRSF (SEQ ID 97)	WP_128450850.1	MGHLLTKKRIKHFEIILKISERCNINCDYCYVFNKGNSDADNNPARISNKNIGHLANFLQRACLEYEIDTLQIDFHGGEPLLMKKEHFANMCIQLISGNYCGSNIRLALQTNGILIDDEWISLFEKYSVNVSLSIDGPKHINDRHRLDTKGRSTYEGTVRGLRLLQSAYQQGRLPSAPGILCVANAQANDAEIYRHFVDDLGVYGFDFLIPDDSYNDVNIDPIGIGRFLNEALDEWVKDNNPKIFVRHFQTHFASLLGVKNIGILGQSSNITGVYAFTVSSDGSIRVDDTLRSTSDRIFNTIGHISEINLSDVLNSPQAQEYSSIGQCLPNECKGCIWENICTGGRLVNRFSSEERFKHKSVYCYSIRSFLSRASAHLLNMGIKEERIMTSICQ (SEQ ID 153)
	WVNAFANWPKRF	1529.72	*Erwinia*	CDE	WP_212410257.1	MKTLKREIERNNCQLTDVDVVTKKAERKALVDGLLDTVSGGWVNAFANWPKRF (SEQ ID 98)	WP_212410258.1	MGANKEKIKHLEIILKISERCNINCDYCYVFNMGNQLATESNPVISMSNILSLRGFFERSVKEYEINVLQVDFHGGEPLMIKKSRFDEMCEILKGGNYSNSKLELALQTNGILIDEEWIVLFEKHKVHVSISVDGPKHINDRHRLDRKGKSTYEGTIKGFRLLQDAWESGRIPGEPGILSVANAKANGAEIYRHFVDVLDCKRIDFLIPDDHHNDEVDSQGIGMFLTEALDEWFSDGNSGVFVRIFNTYLGTMLNHQFSRVLGMSANVESAYAFTVTSDGIIRIDDTLRSTSDKIFDALGHVDEMSLSDVFEHNNFKEYIYLNAVLPAGCHGCLWSNICHGGRLVNRFSLDGRFNNKTIFCSSMKIFLSRAVAHLLASGIEEETIIKNIEKKEISVSDKIFDALGHVDEMSLSDVFEHNNFKEYIYLNAVLPAGCHGCLWSNICHGGRLVNRFSLDGRFNNKTIFCSSMKIFLSRAVAHLLASGIEEETIIKNIEKKEISV (SEQ ID 154)

	WVNAFLNWPRSF	1530.71	*Yersinia*	DEC	WP_072089902.1	MSRLKKEITETKTAIGSNKAKKNQPQHLADDLLDQIAGGWVNAFLNWPRSF (SEQ ID 99)	WP_050317896.1	MDNLLTKKRIKHFEIILKISERCNINCDYCYVFNKGNSDADNNPARISNTNISHLANFLQRACFEYEIDTLQIDFHGGEPLLMKKEHFANMCIQLISGNYRGSSIRLALQTNGTLIDDEWISLFEKYSVNVSISIDGPKHINDRHRLDTKGRSTYEGTVRGLRLLQSAYRQGRLPSAPGILCVANARANGAEIYRHFVDDLGVYGFDFLIPDDSYNDVNIDPIGIGRFLNEALDEWVKDNNPKIFVRHFQTHFASLLGVRNIGVLGQSSNITGVYAFTVGSDGSIRVDDTLRSTSDRIFNTIGHISEINLSDVLNSPQAQEYSSIGQCLPNECKGCIWENICTGGRLVNRFSSEERFKHKSVYCYSIRSFLSRASAHLLDMGIKEERIMAAISQ (SEQ ID 155)
	WVNAFANWTKRF	1533.71	*Aeromonas*	DEC	WP_201910365.1	MSKLQREIALNKTKLINADDKKVERKVLVDSLLDTVSGGWVNAFANWTKRF (SEQ ID 100)	WP_201910362.1	MTLIKGEKIKHLEIILKISERCNISCTYCYVFNMGNSLAADSSPVMSLDNVLALRGFFERSASENEIEVIQVDFHGGEPLMMKKNRFDQMCNILLQGNYGNSRLELALQTNGILIDEEWITLFEKHKVHTSISVDGPKHINDRHRLDRKGKSTYEGTINGLRLLQKAWEQGRLPGEPGILSVANAKANGAEIYRHFVDVLKCQRFDFLIPDDHHDDNTDNEGVGKFLNEALDAWFADGRPELFVRIFNTYLGTMLDNQFSRVLGMSANVESAYAFTVTADGLLRIDDTLRSTSDEIFNAVGHVRDLSLKSVLKNSSVKEYLSLSGELPNDCVDCVWNNVCHGGRLVNRFSKANRFNNKTVFCSSMRVFLSRAAAHLMATGIDERAIMENIQK (SEQ ID 156)
	WVNAFARFTKRF	1536.76	*Vibrio*	DE	WP_083932216.1	MSKLEKEITINNASVSLNKEVKPEKNKDKNELVQSMLDSVSGGWVNAFARFTKRF (SEQ ID 101)	WP_039980110.1	MIRKKIKHLEIILKVSERCNINCTYCYVFNLGNDIAINSKPIISHQNIKHLKHFFERATREYEIESLQVDFHGGEPLMMGKERFKAACKELMSGDYQNSRLSLACQTNAILIDDEWIDIFSKYDVSVGISIDGPKHINDKHRIDRKGRGTYDDTVAGLKKLQAAWEEGKIADEPGILCVANPSVKGADIYRHFVDVLGCKKFDFLIPDESHDTCEDPHSLAEFYCSALDELFNDADKDIYVRYFHTHIHSMLASNFNPVMGMSKSTNDTIAYTVSSEGELYIDDTLRATNDNIFTSIGNIKDLTLSESINSWQMQKYMQVNNQTPEPCSECIWKNICGGGRHIQRYSKEDDFNRNSVYCPSIRKIMSRTASHLISSGIPEEKILTNLGVHN (SEQ ID 157)

	WINVFARWNRAI	1539.76	*Xenorhabdus*	CDE	WP_092519408.1	MSELQREIALNNAQVINSSEKKQERKELVENLMDSVSGGWINVFARWNRAI (SEQ ID 102)	WP_175486043.1	MLTMIKKKKIKHLEIILKVSERCNINCTYCYVFNLGNEISINSKPIISHSTIKDLRAFFEQASQEYDIETIQVDFHGGEPLMMGKEKFENACNEFISGGYNKTKLNLACQTNAILIDNEWIDIFSKYNVSVGISIDGPKHINDKYRLDRKGRSTYEGTVRGLVMLQEAWNAGRLIDQPGILCVANPSVKGAEIYRHFVDVLKCKKFDFLIPDESHDTCANPDGLSDFYCSVIDAFFSDADQDVYVRYFLTHMQSMLSSEFSPVMGLNKSGNDTIALTVSSEGDIYVDDTLRSTNAPIFTSIGNILNLTLSETIASWQMQKYMTVNNQLPTACTDCIWKKVCGGGRHIQRYSKADDFKRESVFCPSIRKIMSRAASHLIESGISEDIIMKNLGIKS (SEQ ID 158)
	WVNVFARWDKQI	1555.76	*Providencia*	D	WP_206277116.1	MSKLSKEIKENNANVKLASNERSSRETLVKSMLESVSGGWVNVFARWDKQI (SEQ ID 103)	WP_206277115.1	MDKIKHLEVILKVSERCNINCTYCYVFNLGNEVAINSKPIISSEIINHLVEFFEQATTEYDIESIQVDFHGGEPLMMGKKRFIAACQKLISGNYNNTKLYLACQTNAILIDPDWIDIFSKYSISIGVSIDGPKHINDKHRLDTKGRSTYDNTIKGFKLLQNAWREGKLKDQPGILCVANPNVSGKDIYRHFVDELECTKFDFLIPDETHDTCIDPTHLSEFYCSALDEFFLDSNNDIYIRYFHTNIQSMLKSDFTPTMGVSKTSNDIIALTISSEGDVYIDDTLRGTNDDIFSVIGNIKKTKFRETLSSWQMEKYMQINSQLPSDCVNCIWKKTCSGGRHIQRYSKADNFNRKSVFCPSIKKILSRAASHLLESGVPEELIMDNLGIKS (SEQ ID 159)
	WVNAFARWDKKF	1561.77	*Sodalis*	CDE	WP_213989265.1	MSKLIKEINFNKAAVTIVADNKNAKKALTQAMLDSISGGWVNAFARWDKKF (SEQ ID 104)	WP_213989266.1	MIKIKHLEIILKVSERCNINCTYCYVFNLGNDISINSKPIISHDIIKDLTGFLERASHEYDIETIQIDFHGGEPLMMGKEKFDSACRDFLSGNYKKSRLQLACQTNAMLIDEEWIDIFSNNNISVGVSIDGPKHINDKHRLDRKGRSTYEGTVKGLVMLQDAWQAGRLIDEPGILCVANSLVNGAEIYRHFVDVLHCKKIDFLIPDETHDTCKDPEGLSDFYCSAIDEFFSDADSNVYIRFFYTHIQSMLNSDLSPVLGLSKSESDTLAFTVGSEGELYVDDTLRATNDPIFTSIGNVRNLSLSETIASWQMQKYMAVNNNLPLVCTDCIWQKICGGGRHIQRYSKADDFNRETVFCPSIRKIMSRAASHLLDCGVSENTIMKNLDS (SEQ ID 160)

	WLNVFVRWDRAI	1568.8	*Xenorhabdus*	CDE	WP_071826505.1	MSKLQREI DLNNAQVI NSSEKKQE RKELVENM MDSVSGG WLNVFVRW DRAI (SEQ ID 105)	WP_196243385.1	MITMIAKKKIKHLEIILKVSERCNINCT YCYVFNLGNEISINSKPIISHNTIKDLR AFFEQASQEYDIETIQVDFHGGEPLM MGREKFENACNEFISGSYNKTKLNLA CQTNAILIDNEWIDIFSKYNVSVGISI DGPKHINDKYRLDRKGRSTYEGTVRG LVMLQEAWNAGRLIDQPGILCVANPS VKGAEIYRHFVDVLKCKKFDFLIPDES HDTCANPDGLSDFYCSVIDEFFSDAD QDVYVRYFFTHMQSMISSEFSPVMGL SKSGSDTIALTVSSEGDIYVDDTLRAT NDPIFTPIGNILNLTLSETIASWQMQK YMTVNNQLPTACTDCIWKKVCGGGR HIQRYSKADDFKRESVFCPSIRKIMSR AASHLIESGISEDIIMKNLGIK (SEQ ID 161)
Xenorceptide A4	WVNAYARWTNRF	1577.72	*Photorhabdus*	DEC	WP_072023203.1	MEESFMSN LKKEIAETK TEIKGTKVK NNQPQPLT EDLLDQISG GWVNAYAR WTNRF (SEQ ID 106)	WP_036768348.1	MVNSLVKKKIQHLEVILKISERCNINC DYCYVFNRGNSAANDSPARISHANID YLVDFFQRGSQEYDIDTLQIDFHGGEP LMMKKPQFASMCERLASGNYHGSKIR FALQTNGILIDDEWISLFEKYSVSVSV SIDGPKHINDRHRLDRKGRSTYEGTI RGLRKLQEAYQAGRLPSDPGILCVAN AKASGAEIYRHFVDNLGVYGFDFLVP DDCYTDAQVDPDGVGRFLNEALDEW VNDNNPKIFVRLFNTHIASLLGAENAG FLGHNPSVAGIYAFTIGSDGFVRVDDT LRSTSDRIFDIIGHISEISLSEVLNSPQ FQEYASIGESLPTECEDCIWAKVCAG GRIVNRFSHEERFKRKSVYCYSMRSLL SRVSAHLLNMGIEEDRIMKAIGR (SEQ ID 162)
	WVNAYARWTKRF	1591.79	*Photorhabdus*	DEC	WP_214085658.1	MSSLKKEIA ETKTEIKGT KVKNNQPQ PLTEDLLDQ ISGGWVNA YARWTKRF (SEQ ID 107)	WP_214085659.1	MVNSLVKKKIQHLEVILKISERCNINC DYCYVFNRGNSAANDSPARISHANID YLVDFFQRGSQEYDIDTLQIDFHGGEP LMMKKQQFASMCERLASGNYYGANI RFALQTNGILIDDEWISLFEKYSVSVS VSIDGPKHINDRHRLDRKGRSTYEGT VRGLRKLQEAYQEGRLPSDPGILCVA NAKASGAEIYRHFVDNLGVYGFDFLV PDDCYTDAQVDPVGVGRFLNEALDE WVNDNNPKIFVRLFNTHIASLLGAEN AGFLGHNPSVAGIYAFTIGSDGSVRV DDTLRSTSDRIFDIIGHISEISLSEVLN SPQFQEYSSIGESLPTECEDCIWAKVC AGGRIVNRFSNEERFKRKSVYCYSMR SLLGRVSAHLLNMGIEEDRIMKAIGR (SEQ ID 163)

	AGWINAFGNWTKSF	1592.73	*Yersinia*	DEC	WP_072080131.1	MSRLKKEITATKTVINVNEVKKSQPQRLAEDALEQITGGAGWINAFGNWTKSF (SEQ ID 108)	WP_050143454.1	MVELLINKRIRHLEIILKISERCNINCDYCYVFNKGNSAANDSPARISDKNIHHFVNFLERASQEYQIGTLQIDLHGGEPLLMKKENFANMCIQFMSGHYCGSNIRLALQTNGTLIDEEWIALFERYSVNVSVSIDGPKHINDRHRLDTKGRSTYEGTVRGLRMLQQAYQQGRLPSAPGILCVANAKVNGAEIYRHFVDDLGVYSFDFLIPDDCYKDADVDSLGLGRFLNEALDEWVKDDNPKIFVRLFQTHIATLLGQKNSGILGHNPSVTGVYALTVSSDGFVRVDDTLRRSTSDSMFNPIGHTSEVSLSEVFDSPQFREYTSVGQSLPTECTGCIWENICAGGRIVNRFSPEDRFDRKSAYCYSMRSFLSRASAHLINMGIKEERIMAAISQ (SEQ ID 164)
	AGWINAFANWTKSF	1606.76	*Yersinia*	DEC	WP_071984814.1	MSRLKKEITATKTVINVNEVKKSQPQRLAEETLEQIAGGAGWINAFANWTKSF (SEQ ID 109)	WP_050538194.1	MVELLIDKRIRHLEIILKISERCNINCDYCYVFNKGNSAANDSPARISDKNIHHFINFLERASQEYQIGTLQIDLHGGEPLLMKKENFANMCIQFMSGHYCGSNIRLALQTNGTLIDEEWIALFEKYSVNVSVSIDGPKHINDRHRLDTKGRSTYEGTVRGLRMLQQAYQQGRLPSAPGILCVANAKVNGAEIYRHFVDDLGVYSFDFLIPDDCYKDADVDALGLGRFLNEALDEWVKDDNPKIFVRLFQTHIATLLGQKNSGILGHNPSVTGVYALTVSSDGFVRVDDTLRSTSDSMFNPIGHTSEVSLSEVFDSPQFREYTSVGQSLPTECTGCIWENICAGGRIVNRFSPEDHFDRKSAYCYSMRSFLSRASAHLINMGIKEERIMAAISQ (SEQ ID 165)
	AGWIKAFGNWSRSF	1620.79	*Yersinia*	DEC	WP_072088965.1	MSRLQKEIIETKTVIDVSGAKKSQPQRLTEDVLEQIAGGAGWIKAFGNWSRSF (SEQ ID 110)	WP_050291264.1	MLNLLIEKNIRHLEIILKISERCNINCDYCYVFNKGNSAADDSPARLSNKNIHHLVCFLQRACQEYKIGTVQIDFHGGEPLLMKKENFTDMCIQLISGNYCGSNIRLALQTNATLIDNEWIAIFEKYSVNVSISIDGPKHINDRHRLDTKGRSTYESTVRGLRILQNAYQQGRLPSDPGILCVTNAQANGAEIYRHFVDELGVYSFDFLIPDDSYKDAHPDAVGIGRFLNEALDEWVKDDNAKIFVRLFQTHIASLLGQKNSGVLGHTPNITGVYALTVSSDGFVRVDDTLRSTSDRMFNPIGHLSEVNLSNVFASPQFQEYSSIGQSLPTECEGCIWENICAGGRIVNRFSTEDRFKHKSIYCYSMRTFLSRSSAHLLNMGIKEERIMAAIRA (SEQ ID 166)

	WVNAFARWSRRW	1628.82	*Serratia*	CD	WP_072056064.1	MSKLAKEISMNKAAVIIDGDKKDIRRALTQSMLDSISGGWVNAFARWSRRW (SEQ ID 111)	WP_072056065.1	MANKEKIKHLEIILKVSERCNINCTYCYVFNLGNDLAINSKPIISHGVIKNLREFFERACREYEIETVQVDFHGGEPLMMGKDRFDNACKELVSGDYNGTRLNLACQTNAILIDNEWIDIFSKYNMSVGISIDGPKHINDRHRLDRKGRSTYEGTVKGLEMLQVAWRAGRLIDEPGILCVANPSVKGAEIYRHFVDVLKCKKFDFLIPDESHDTCTDPEGLSDFYCSALDEFFLDADKEVYVRYFHTHIQSMLSSEFSPVMGVSKAGSDTLAFTVSSDGELYVDDTLRSTNDSIFTPIGNLHSLTLSEALMSWQMQKYLSVDNQLPKVCIDCVWKKLCGGGRHIQRYSSNDDFNRETVFCPSIRKIMSRAASHLIESGVSEDVIMKNLEVNS (SEQ ID 167)
	AGWINAFANWTRSF	1634.77	*Yersinia*	DEC	WP_072079580.1	MSRLKKEITATKTVINVSDVKKSQPQRLAEDALEQIAGGAGWINAFANWTRSF (SEQ ID 112)	WP_099466089.1	MVETLIDKRIRHLEIILKISERCNINCDYCYVFNKGNSAANDSPARISDKNIRHFVDFLERASQEYQIGTLQIDLHGGEPLLMKKENFANMCIQFMSGYYCGSNIRLALQTNDTLIDEEWIALFGKYSVNVSVSIDGPKHINDRHRLDTKGRSTYEGTVRGLRMLQQAYQQGRLPSAPGILCVANANVNGAEIYRHFIDELGVYSFDFLIPDDCYKDTYVDAVGMARFLNEALDEWVKDNNPKIFVRLFQTHIATLLGQKNSGILGHNPSVTGVYALTVSSDGFVRVDDTLRSTSDPMFNPIGHTSEVSLSEVFNSPQFQEYSSIGQSLPTECAGCIWENICAGGRIVNRFSPEDRFDRKSAYCYSMRSFLSRASAHLINMGIKEERIMAAISQ (SEQ ID 168)
Xenorceptide A1	WINAFGNWERAFH	1641.77	*Xenorhabdus*	CDE	WP_010848441.1	MSKLQREIAANKAQLSHEDKKKTQHKELVDSLLDTVSGGWINAFGNWERAFH (SEQ ID 113)	WP_010848442.1	MTTSKSEKIKHLEIILKISERCNINCSYCYVFNMGNSLATDSPPVISLDNVLALRGFFERSAAENEIEVIQVDFHGGEPLMMKKDRFDQMCDILRQGDYSGSRLELALQTNGILIDDEWISLFEKHKVHASISIDGPKHINDRYRLDRKGKSTYEGTIHGLRMLQNAWKQGRLPGEPGILSVANPTANGAEIYHHFANVLKCQHFDFLIPDAHHDDDIDGIGIGRFMNEALDAWFADGRSEIFVRIFNTYLGTMLSNQFYRVIGMSANVESAYAFTVTADGLLRIDDTLRSTSDEIFNAIGHLSELSLSGVLNSPNVKEYLSLNSELPSDCADCVWNKICHGGRLVNRFSRANRFNNKTVFCSSMRLFLSRAASHLITAGIDEETIMKNIQK (SEQ ID 169)

	AGWIKVFGNWSRSF	1648.84	*Yersinia*	C	WP_071881823.1	MKKEIIETKTVIDVSDTKKNRPQHLAEDVLEQIAGGAGWIKVFGNWSRSF (SEQ ID 114)	WP_042661398.1	MLNLLIEKKIRHLEIILKVSERCNINCDYCYVFNKGNSAADDSPARISNKNIHHLVYFLQRACQEYQIDTIQIDFHGGEPLLMKKESFTNMCIQLISGNYCGSQLRLALQTNATLIDNEWIAIFEKYSVNVSISIDGPKHINDRHRLDTKGRSTYEGTVRGLRILQHAYKQGQLPSDPGILCVANAQANGAEIYRHFVDELGVYSFDFLIPDDSYKDAHTDAIGIGRFLNEALDEWIKDNNAKIFVRLFQTHIASLLGQKNSGVLGHTPNVTGIYALTVSSDGFVRVDDTLRSTSDRMFNPIGHLSEVNLSNVFASPQFQEYSSIGQSLPTECEGCIWENICAGGRIVNRFSTKDRFKRKSIYCYSMRTFLSRSSAHLLNMGIKEERIMAAIQA (SEQ ID 170)
	WVNVFARWSRRW	1656.87	*Serratia*	CDE	WP_103774054.1	MSKLAKEISMNKAAVIIDGDKKDVRRALTQSMLDSVSGGWVNVFARWSRRW (SEQ ID 115)	WP_103774053.1	MANKEKIKHLEIILKVSERCNINCTYCYVFNLGNDLAINSKPIISHGTIKNLRGFFERACQEYEIETVQVDFHGGEPLMIGKDRFDNACKELVSGDYNGTRLNLACQTNAILIDNEWIDIFSKHNISVGISIDGPKHINDRHRLDRKGRSTYEGTVKGLEMLQAAWRAGRLIDEPGILCVANPSVKGAEIYRHFVDVLKCKKFDFLIPDESHDTCTDPEGLSDFYCSALDEFFLDADKEVYVRYFHTHIQSMLSLEFSPVMGVSKAGSDTLAFTVSSDGELYVDDTLRSTNDSIFTPIGHIQSLTLSEALTSWQMQKYLSVDNQLPEVCIDCIWKKLCGGGRHIQRYSSADDFNRETVFCPSIRKIMSRAASHLIESGVTEDIIMKNLEVNS (SEQ ID 171)
	AGWIRAFANWSRSF	1662.83	*Serratia*	DEC	WP_023489715.1	MTRLKKEIIETKTMIDVNSVKNNQPQHLTEDVLDQISGGAGWIRAFANWSRSF (SEQ ID 116)	WP_037383507.1	MVNLLNKKHIKHLEIILKISERCNINCDYCYVFNKGNSASNDSPARLSDKNVNHLVDFFQRACLEYEIGTLQIDFHGGEPLLMKKENFDRMCDRLVTGNYCGSNIRLALQTNGMLVDDEWLALFEKHSVNVSISIDGPKHINDRHRLDTKGRSTYEGTVRGLRKLQHAYQQGRLPSDPGILCVANAQANGAEIYRHFVDDLNVRSFDFLIPDDCYKDTHVDPVGLGRFLNEALDEWVKDDNAKIFVRLFQTHIASLLGKENVGVLGHTPSITSVYALTVSSDGFVRVDDTLRSTSDRMFNTIGHLSEINLSDVFDSPQFQEYASIGQSLPTECKGCIWENICAGGRIMNRFSTEERFKRKSVYCYSMRSFLSRASAHLLNMGIKEERIMEAINR (SEQ ID 172)

	WFRAYLRWSRSF	1668.88	*Mixta*	DC	WP_165786503.1	MNFTINDLK KLLLNTEEN RSPSVAKET IEELSNDDL TNVGGGWF RAYLRWSR SF (SEQ ID 117)	WP_103059455.1	MAKKIDILEIILKVTECCNIACRYCYYF EGDNRDFADKPRVMNKKTVIQLANYL KETVVAHQIETLRIDIHGGEPLMMGK KRLGELLLILSDALKKICKLEFVLQCNG TLIDDDWINIFAKYQVAASVSVDGDA VTHNLNRIDRRGKGTYHRVMAGLSKL IAASKDNKVPYPGVLCVINPDKNGKVI FRHFVEQNKTPYISFIEPDFTIDEASKQ RVDGIGNFLLDVYQEWEKNNSPKINR HMSLRVFNDLLSVLMVSGTEYENMKT INYVVITIRSDGYINPDDILRNTHPELF NESYHLASSTLEEFITSEDIRELYRGIF TLPVQCQECGVRKLCRNGFCFGSLPH RYSKKNGMNNTNLFCKFYREICIRLCN YAVNKGKTFAEIEKAVY (SEQ ID 173)
	WWRAYARWRRSF	1734.95	*Gilliamella*	DEC	WP_160406027.1	MFFSKKTIE QRLRDTEA KRKNVPNA KAMEELAA QYLDEVNG GWWRAYA RWRRSF (SEQ ID 118)	WP_160406026.1	MSNSIKVDILEVILKITECCNIACRYCY FFRGGNIDFDERPNVIKKDTIHALASF LKEAILANEIKLLRLDFHGGEPLMMGK KRFVEMVELFDTELSQLVDLEYVLQSN GTLIDDEWVEIFSKYNVAASVSLDGD QAIHDANRIDKKGRGTYVRATEGLKK LICAARSNKVVFPGIISVINDSSDTKIT FKHFLDDLESPFISFVELDLTIDELNQE TVEKISNNLLAVYNEWERINTPTIVHD ISVRNFNDILKQLVLSGTEADKKEKRK YVSLTIRSDGSLNPDDILRNIYPYLFTN EYNIKNNTLSDYLSDEKLKDLYRKLFT LPEKCNECGVKKICRNGWGFGSIPHR YSKENDMNNVNALCGVYHEISLRLCD LVIQQGKSYDSIKHNLF (SEQ ID 174)
	DRWLKWIKNH	1391.6	*Photorhabdus*	CDE	WP_181147865.1	MSKLAKEIK ENKTTVTTK KSADQKAM AQSLLDNV CGGGDRW LKWIKNH (SEQ ID 119)	WP_219847460.1	MKKIKHLEIIAKVSERCNINCTYCYVF NMGNDLAINSKPVISLKTVSNLKRFLE RSLTEYNIESIQVDLHGGEPLMLNRER FSRMCEELMSGDYKGAKFSIACQTNA TLIDDEWIDIFSKYNISVSVSIDGPKHI NDKNRIDNKGKGTYDATVSGLFKLQS AWKDGKLPSAPGVLCVANPNSNGAE VYRHFVDVLNCKSFDFLIPDESHDNC KNPYGISDFFCSAVDEFFSDADKKIIV RYFYATIQGMLNPGIFHVAGMGKMNN DIVAFTMGSEGNIHVDDILRSSNDDIF TAIGNVNELSLNNVI (SEQ ID 175)

	DGRWLQWIKNH	1448.61	*Kosakonia*	CDE	WP_180344379.1	MKKLAKEVKQNGVSVNTAKNKAQKKFSQSLLDDVQGGDGRWLQWIKNH (SEQ ID 120)	WP_139569738.1	MKSIEHLEIIVKISERCNIDCTYCYVFNKGNDLAINSQTIIKKNTINSFRDFLESASKGFDIKTIQIDFHGGEPLLLKKDRFNFLCKTLREGDYRGSRLVLSCQSNGVLIDDEWIDIFHKWDVGVSVSMDGPKHIHDAARIDKNGKGTYDQVVAGFRKLQDAWKENKISTQPGILCVANTNLKGVEIYRHFIDDLQCKGFDFLIPDETHDSNIDASKLYDFYESVIDEYFIDADIDIKFRYLKVLIQGMLNPGTYAIAGLNAVNNDIVALTMGANGDIYIDDTLRSTSDKAFSKIINISSGSLGDILSSWQYLEYTKFANTLPIECETCTWKKLCGGGGLVQRYSKEQRFNGKSVYCHSLKKIYGRVASHLIESGIDETHILKSLGCNDGN (SEQ ID 176)
	WVNAFLN	858.95	*Yersinia*	DEC	WP_072086462.1	MSRLKKEITETKTAIGTNKAKKNQPQHLADDLLDQIAGGWVNAFLN (SEQ ID 121)	WP_050097262.1	MGHLLTKKRIKHFEIILKISERCNINCDYCYVFNKGNSDADNNPARISNKNIGHLANFLQRACLEYEIDTLQIDFHGGEPLLMKKEHFANMCIQLISGNYCGSNIRLALQTNGILIDDEWISLFEKYSVNVSLSIDGPKHINDRHRLDTKGRSTYEGTVRGLRLLQSAYQQGRLPSAPGILCVANAQANGAEIYRHFVDDLGVYGFDFLIPDDSYNDVNIDPIGIGRFLNEALDEWVKDNNPKIFVRHFQTHFASLLGVKNIGILGQSSNITGVYAFTVGSDGSIRVDDTLRSTSDRIFNTIGHISEINLSDVLNSPQAQEYSSIGQCLPNECKGCIWENICTGGRLVNRFSSEERFKHKSVYCYSIRSFLSRASAHLLNMGIKEERIMTSICQ (SEQ ID 177)
	FANASWPKSF	1150.26	*Bordetella*	CD	WP_176463924.1	MMTKEIIQHLEQVQRNAAEEEKTVEEISQSELDQICGAGGVGGFANASWPKSF (SEQ ID 122)	WP_176463923.1	MHYIEIILKVAERCNLNCTYCYFFNKENKDFEDHPALISPDTVRQLVQFLRTSSHEISETVFQIDIHGGEPLLLGPRRFSEMVSIIENGLQDAKEVRFTVQTNAVLINDAWLDVFSRHKVFVGVSVDGPKDRHDANRIDRRGRGTFDSMVPKIAALKQATSEARIPGFGSISVVSPESNGRATYTCLTQELGFSKLQFLFPDDTHDSANPANAGRFISFVDDLFECWEEDNSRDVRIKFIDQTLVALLQNKHYIQRGRRVNPAFEGVVFTVSSAGDIGHDDTLRNVAPELFKSGMNVANAKFPEFIAWHNMVSGILVSPDLPAPCASCAWNNICEHVTGSYTPLHRMKNGTADQPSVYCEALKVAYQRGAEYLAKRGHPIHQISKNLNPA (SEQ ID 178)

	FANATWSKSF	1154.25	*Bordetella*	CDE	WP_156770205.1	MTTKEIIQH LEQVQRNA AQEEKQME EISQEELEK ICGAGGVG GFANATWS KSF (SEQ ID 123)	WP_082993604.1	MHYVEIILKVSERCNLNCTYCYFFNKE NRDFEGHPALISPNTVRHLVRFLRTSP HQISETVFQVDIHGGEPLLLGPKRFSE IVSIIENGLSDAKEVRFTVQTNAVLINE AWIDVFAQHKIFVGVSVDGPKGQHD ANRIDRRGRGTFDSMVPKIAALKQAA LERRIPGFGSISVVSPALDGRATYICLT KELHFAHLQFLFPDDTHDSTNPALAEG FAKFVEDLFASWQSDGNDNIHIKLID QTLLGFLQDKQYIDGGRRISPAVGRV VFTVSSAGDIGHDDTLRNVAPELFKS GMNVSDANYAEFIVWHNRVSKILFPR DLAPPCASCAWNNICEHVTRSYTPLH RMKDGRVDQPSVYCEALKTAYRNGA EYLAKRGLPIREISKNLNPDY (SEQ ID 179)
	FANATWPKSF	1164.29	*Bordetella*	CDE	WP_157664463.1	MMTKEIIQH LEQVQHNA AEEEKPIEEI SQSELDQI CGAGGVGG FANATWPK SF (SEQ ID 124)	WP_086057504.1	MAINHGEHATMPYVEIILKVAERCNLN CKYCYFFNKENRDFEDNPALISPNTVR QLVQFLRTSSHEISETVFQIDIHGGEP LLLGPRRFSEMVSIIENGLHDAKEVRF TVQTNAALINDAWLDVFSRHKVFVGV SVDGPKDQHDANRIDRRGRGTFDTM VPKIAALSQATSQGRIPGFGSISVVSP ESDGRATYMCLTKELRFSKLQFLFPDD THDSANTKNAGRFIKFVGDLFECWEN DNNRDVRIKLIDQTLAAFLQDKHYVE AGRRVNSAAQGVVFTVSSAGEIGHD DTLRNVAQELFRSGMNVADAKYPEFL AWHNMISGMLVPRDLPPPCASCAWN NICEHVTGSYTPLHRMKNGTADQPSV YCEALKIAYRRGAEHLAKRGVPIHRIS KNLTPVQRATS (SEQ ID 180)
	WVNFQWKNSW	1390.52	*Providencia*	CDE	WP_210852630.1	MKKFKTVIQ ENSANLKIK KDSDVSKL LEHIRGGKS EAAGGWVN FQWKNSW (SEQ ID 125)	WP_210852632.1	MLKIKHFEVILKISERCNLNCTYCYIFN MGSELALNSAPVISNTTIVELKNFLER VADEVEHNVIQVDLHGGEPLMLKKKR FIYLCETLRSGDYKGAEFRIGLQTNAT LIDDEWLEIFEKYNISVSISIDGPKHIN DRYRLDHKGRSSYEATMNGYQALYSA AENRKIIPTPPILSVINPDASGKELFEY FYHDMKCRKFDFLLPDNNYVNTVDTE GIKRFLVDICDAWFAQNDPECDIRILS AYLRILTGAEDYIVLGVTPQNELHQTIA ITVTSTGYIYVDDTLRSTLSDIFVPICH IRDASYQKIITSFPMRELSKIESFLPDD CHGCIWKAVCAGGRPINRYSQDNAF KNKTIYCDAMQSFLSRGAAYLINLGIN SNEIAKNIGIDKNA (SEQ ID 181)

	NVFVNATWSRAM	1391.57	*Pandoraea*	CDE	WP_157122607.1	MTTKAFIEQ LAKKQKAA NEAGSIKEI PASELERIS GARGGNVF VNATWSRA M (SEQ ID 126)	WP_046290456.1	MKQYVEVILKVSERCNIDCKYCYFFNK ENKDYASNPPYMTQQTAEDFVTFLRS SPNLRETTFQIDLHGGEPLMMKRERFE ALVTTLKNGLSDAESVQFTVQTNAML VDEAWLDLFSRLGVYIGVSIDGPKIYH DENRVDKQGMGTYDRTVEKIALIKAA ADTGLISGFGAICVMNPKFDARLVYDT LTRTLGIYNLQFLLPDESHDSVRTADV MALKWFTQALFDCWADDPRGTVRIR SIDRMLDAILADEPRKDVIWRDARSS VVFTLSSGGDIGHDDTLRNVIPDVFYA RMNVASSTFSEFLAWHATVSAMLARR TTAVACRTCLWREICEIATRSDTPLHR CKNGVADQHTVYCECLKANYEKGAEY LALSGVAIEEISRNFVEVD (SEQ ID 182)
	WSRTVFNRVRPV	1512.74	*Erythrobacter*	DEC	WP_212451268.1	MAKNKTPK TEAKAQSK SLESLIDAQ LDSIVVGG WSRTVFNR VRPV (SEQ ID 127)	WP_212451270.1	MFDVEARLARPGRRHVSVVLKVAERC NLACTYCYFFFGGDDSYLKHPALISSD RVSDVARFLGEAAIKHRLERIEIALHG GEPLLLKPDRMGALVETIRAAVPDSCE VDILLQTNGVLVDETWIALFEQHSIGI GVSLDGPRAVNDIARLDKKGRSSFDA TIAGWGLLKKAAADGRISEPGILSVIA PTTDAETLSFFIDELGAHSLNFLLPDMF FDNPETQPEDVARIGETMIAIFEEWRR RADPGLHIRFVNDALLPMIVAIPAEST HHCREDLSHAMTIASDGTIYVEDTIRS AFADRFDETLNVASATLADVFAHPHW QSIARAAEQPAGPCTSCRYGEICQGG PLISRYSSDRGFDNPSLYCSALFAFHR HVEREVSATGRLLPSPRFAADPLFPAR KEVA (SEQ ID 183)
	AGNDGWVKFGWKKKF	1764.02	*Sodalis*	CDE	WP_213990087.1	MDKLRDAI KNNTKTPLA KDTGDLLK SIRGGAGN DGWVKFG WKKKF (SEQ ID 128)	WP_213990088.1	MKDKQPKHLEIILKVSERCNLNCSYCY VFNMGSDLALNSAPVISRATINSLKNF LERSVREYSIDVIQIDLHGGEPLMLKK ERMAVLCALIREGDYNGASVQIGIQT NATLIDEEWIEIFSRYHVSVSISIDGPK HVNDIHRLDHQGRSSYEKTLRGYKLL STRSTDGKKEINAPVLSVLTPKANGSE LFSHLYDVMGCRNFDFLLPDCNYDNPI DTAAIGRSLIEICDKWYAQNDPDCVV RIVNAHMAHLAGNKKNVVLGVTNVN KNALALAFTVTSQGEIYVDDTLRSTHS DIFTSIGNITHTSLEEIFASRQLIALNII QDTIPRECSECVWRNICAGGRPINRY SSIDGFTGKTIYCDAMKMFLGRCASIL NEMGVSIEELVINLGIENDK (SEQ ID 184)

	RGEGWVRAYWAKRF	1778.01	*Kosakonia*	CDE	WP_139569744.1	MSKLAKEIASNKATVTTPTAKAAHVANLLDNVQGGRGEGWVRAYWAKRF (SEQ ID 129)	WP_139569743.1	MRTKIKHLEIILKVSERCNINCTYCYVFNLGNELAINSKPVISASTIGDLRRFLENAAIEHGIETLVIDFHGGEPLMMGKKKFAAACEVFRSGNYGNGELHLACQTNGILIDDEWIDLFSKYGVGVGVSIDGPKHINDKHRLDHKGRSTYEGTVKGFRLLQAAYAAGKLELEPGILSVANPFVKGSEIYRHFVDTLNCKRFDLLIPDESHFSCKNPNEIADFYCSAIDEFFFDGNPDINIRYINTHVQAIVSNNHAQTLGVSKSTSDAIAITVMSDGDIYIDDTLRSTNDELFSPIGNVREISFSGVKESWQFKKSAHIANNPPADCKDCLWKKVCGGGSMIQRYSKEEGFERKSVYCPSIKKIFSRMTSHLISAGIPEEKISKNLEG (SEQ ID 185)
	RGQGYVRFIFRRSF	1785.04	*Bartonella*	-	WP_008038584.1	MSKLKSEINTNNHNNAADDLVELSEATIKKLDAAGGRGQGYVRFIFRRSF (SEQ ID 130)	WP_008038586.1	MSNVASKLNVLEIILKLTERCNLNCTYCYVFNKGDYDETSSQALISDNSVNDVIDFVLNAIESYELKLVRIIFHGGEPLLYPKKKFDNLCNSLKALESVDTSITLSLQTNGVLIDETWVEIFSRHDVTVGISLDGNKEMNDQYRLDKKGRSSYERSIKGLRLLQESYNQNKFSHSPSILMVANCENDIDTLYDHVFNNLGVSSFDILLPDDNYLDESRPSDDLMGKYFTRLLDLYLNDERDVFIRLFDAPIYILNSNSMDFLGFSARVHKMMVSLTINTDGLLYVNDVLKPTGAYLASAIGNIKDFKLEDFMASQQYKMYISATEYVPSECQDCIWRNPCSGGALQNRYSKENGFSNKTIYCGTNRSILSRVSEYLIIKGVDESKIMSNIGL (SEQ ID 186)
	KPGEGWVNFTWNKSF	1792.97	*Photorhabdus*	CDE	WP_172911276.1	MKELQKAIQKNSANLKNQKAKEASNLLDAVRGGKPGEGWVNFTWNKSF (SEQ ID 131)	WP_172911275.1	MPKIKHFEVILKISERCNLNCSYCYVFNMGSELALNSAPVISHNTIIELKYFLERVAEETTPDVIQIDLHGGEPLMLKKERFVYLCETLRSGDYKNAEFRLGLQTNATLIDDEWIEIFEKFEVAVSISIDGPKHINDKYRIDHKGRSSYEATLNGYQALYTAAKKRNILPLPPVLSVIDPEANGKELFEHLYHDMQCRKFDFLLPDYNYENPTNTEGIKRFLTAICDAWFEQNDPACDVRILSAHLTRLMGTTGHVILGVTPQIESYKAVAITVTSTGDIYIDDSLRSTLSKIFTPIGNIKNTSYAQIVNSPPMRELSKIEASLPDDCQGCIWKTICAGGRPINRYSRDNAFNNKTIYCDAMQAFLGRGAAYLVELGLSENEIEKNIGIAEHE (SEQ ID 187)

	WVNAFANRTMGFLFKL	1911.25	*Erwinia*	CDE	WP_168428711.1	MSKLQREITSNKAQLVNADVRKMQRKVFVDSLLDTVSGGWVNAFANRTMGFLFKL (SEQ ID 132)	WP_168428712.1	MRLIKGEKIKHLEIIFQVSKRCNISCSY CQVFIMGNTLAADSHPTKSLNNVIALR GFFERSTAENEIEVIQVDFHGGKPLM MKKDRFDQMCHILLQGDYGNSRIELA LQTHGILVDEEWITLFEKYKVQASIPV DGLRHSNNRHRPDRTGESTYKGTING LRLLQNAWQQGRLPAEPGILSVANAK ANGADIYHHFVDVLKCQRFDFLIPDD HHDDITDSEGIGRFLNEALDAWFADG RPELFVRIFNTYLGTLLDKQFSRVLGM SANVESAYAFTVTADGLLRIDDTLRST SDEIFNPVGHVRDLSLAGVLKNTAVEE YLSLSNTLPEGCKDCVWNNVCHGGRL VNRFSQANRFNNKTVFCSSMRIFLSR GASHLMATGIDERTIMANIQG (SEQ ID 188)
	ASTAETWFKLDWKKSF	1941.17	*Xenorhabdus*	DEC	WP_189757993.1	MKELQKIIHENSANLKNQKGQKASELLDFVRGGASTAETWFKLDWKKSF (SEQ ID 133)	WP_189757994.1	MNKINHLEVILKISERCNLNCSYCYVF NMGSDIALNSAPVISHNTIIGLKGFLE RVAEDVNPDVIQIDLHGGEPLMLKKE RLIYLCETLNSGDYKGAELRFALQTNA TLINNEWIAIFEKFNISVNISIDGPKHI NDKYRIDHKGRSSYEATLNGYKALCT AAKERNILNYPSILSVIDPEASGKELFD HFYHDMQCKRFDFLLPDSNYENTTNT EGVKRFLIDVCDAWFEQSDPNCDVRI LSSYFTRLAGSSKYIVLGVTPPTEGFEA LAITVTSTGDIYIDDTLRSTVSEIFTPI GNIADATYAQIVNSQPMREFHKIESSL PVDCQGCIWQKICAGGKPVNRYSRD NAFNNKTIYCDTMAALLGRGAAYLVEL GLSENELAKNIGIAEL (SEQ ID 189)
	SSDDDGIFFKTTWDRR	1942.03	*Xenorhabdus*	DEC	WP_189757997.1	MKELQKVIQENSANLKNQKGQKASELLDAVRGGSSDDDGIFFKTTWDRR (SEQ ID 134)	WP_189757994.1	MNKINHLEVILKISERCNLNCSYCYVF NMGSDIALNSAPVISHNTIIGLKGFLE RVAEDVNPDVIQIDLHGGEPLMLKKE RLIYLCETLNSGDYKGAELRFALQTNA TLINNEWIAIFEKFNISVNISIDGPKHI NDKYRIDHKGRSSYEATLNGYKALCT AAKERNILNYPSILSVIDPEASGKELFD HFYHDMQCKRFDFLLPDSNYENTTNT EGVKRFLIDVCDAWFEQSDPNCDVRI LSSYFTRLAGSSKYIVLGVTPPTEGFEA LAITVTSTGDIYIDDTLRSTVSEIFTPI GNIADATYAQIVNSQPMREFHKIESSL PVDCQGCIWQKICAGGKPVNRYSRD NAFNNKTIYCDTMAALLGRGAAYLVEL GLSENELAKNIGIAEL (SEQ ID 190)

	ADSQPKARAWFANASFSKRF	2281.52	*Burkholderia*	CDE	WP_175425513.1	MDLHVFKK EMMAGAQ QEERELLAE IDPELLALV GGGADSQP KARAWFAN ASFSKRF (SEQ ID 135)	WP_175425514.1	MIEHDKINRLEVILKVTERCNIDCTYC YYFNGNNRDYMGQPPYLTVDTAKSLA VYLRNAACSHSIDEIRIDLHGGEPLLM KKAKMSAVLEILRSGVADFTDLTICIQ TNATLLDEEWISIFEKYSVSVGVSLDG SPDENDLYRVDKKGKGTHSVVVKAIE LLKAANKKSEGIFAGIICVVNPDFDGK KIYRHFVDDLGVERIHFLKANQTRDG ADIKLVAGTRKFLLGALNEWINDGNF NIYVRQFTEPLKQLCTSSAPSPCSDRY VAMTVRANGDIAIDDDFRNTLPSLFNL GLNISDSALADFLDRPGVADFHRACG EVSPSCLQCGAREICKNGTGLAESVL HRYSFINKFRNASLFCESHQAIIIRLG QFAISRGVPWSTIERNMAGIRNN (SEQ ID 191)
	VESQSKPRAWFANSSFSKRF	2355.6	*Trinickia*	CDE	WP_207004678.1	MDLHVFKK EMMAGAQ QVEREMPA ELDPEFLAL VGGGVESQ SKPRAWFA NSSFSKRF (SEQ ID 136)	WP_207004679.1	MLIRLVIQKTPHFLVRNFRGCSTHQCF PKCIEPESSSCVLINNWRRNDGARKI NRLEVIVKVTERCNIDCTYCYYFNGEN GDYANQPPYLTVDTARSLAIYLHNASR SHSIDEIRIDLHGGEPLLMKKTRMSV MLEIFRSSIPDSTDLTICIQTNAILLDE EWISIFAKYNVSVGVSLDGPPRENDLY RVDKKGRGTHSAIAKAIEMLKKANKK CAGVFAGVICVVNPDFDGRKVYRHFV DDLGIERIHFLKPNQTRDGADIKLVEG TSKFLLDALNEWINDSNPNIYVRQFTD PIRRLCASGPSSPFSDRYVAVTVRANG EIAIDDDFRNTLPSLFNLELNVADSAL ADFLNHPGVFDFHQACAEVPPSCLQC GANGICQSGIGLNESVLHRYSFINKFR NASLFCQSHQAIIIRLGQFAISHGVPW STIEKNMIRIHDN (SEQ ID 192)
	ASSQANSRGWFANATWSKAWR	2378.55	*Burkholderia*	CDE	WP_162999177.1	MDLHAFKN EMMVGAQ QVEREAPV ELDSELLAL VGGGASSQ ANSRGWFA NATWSKA WR (SEQ ID 137)	WP_121856868.1	MFISFSTKSHVTSLLARKLAPRNDASL GHQFWTESTLLKISKEMKNIDKINRLE VILKVTERCNIDCTYCYYFNGSNHDYT SQPPYLNIDTAKSLAGYLRDATRAHSI DEIQIDLHGGEPLLMKKSRMSDMLEIF RNSISDQTDLRISIQTNATLLDEEWLS IFAKYNVSVGVSLDGPPRENDLHRVD KKGNGTHSAVSKAIAMLIEKNKTCEG VFAGVICVINPDFDGSKTYRHFVDDL GIERIHFLKPNQTRDAADIKLTEGTSK FLLDTLSEWINDSDRNIYVRQFTDPLK RICASDASESPPHRFVAMTVRANGEI AVDDDFRNTLPSLFNLGLNVSNSTLA DFINHPKVADFHRACDEVPPFCSQCG AKGICQSGAGLGESVLHRYSFINKFR NASLFCTSHQAVIIELGKFALSHGMP WATIEENMTGNRI (SEQ ID 193)

[a]C-terminal residues after the GG motif.

[b]Molecular weight of the fully modified core peptide.
[c]Topology of xyeCDE genes in the biosynthetic gene cluster.
[d]Protein ID and sequence for a representative pair of precursor and rSAM are shown.

The protease, transporter and protease/transporter may be fused or may be separately expressed. In some embodiments, the protease, transporter and the protease/transporter are encoded by the same nucleic acid molecule. In some embodiments, the protease, transporter and protease/transporter are derived from *Xenorhabdus nematophila (Xnc).*

In some embodiments, an amino acid sequence of the protease is at least 70% identical to the amino acid sequence of SEQ ID NO: [XncC]. In some embodiments, an amino acid sequence of the transporter is at least 70% identical to the amino acid sequence of, SEQ ID NO: [XncD]. In some embodiments, an amino acid sequence of the protease/transporter is at least 70% identical to the amino acid sequence of SEQ ID NO: [XncE].

In some embodiments, the protease and/or the protease/transporter is capable of cleaving the modified precursor polypeptide to form the polypeptide. In some embodiments, the protease and/or the protease/transporter is capable of cleaving the modified precursor polypeptide at a Gly-Gly motif.

In some embodiments, the transporter and/or the protease/transporter is capable of transporting the polypeptide out from of a host cell.

In some embodiments, the nucleic acid sequence is provided to the host cell via a phage.

In some embodiments, the method comprises b) isolating the cleaved modified polypeptides that are exported out from the host cell. In some embodiments, the method comprises isolating the polypeptide from the culture medium.

The method may be performed under anaerobic or oxygen-free conditions.

Table 8 shows a list of precursor polypeptide and rSAM sequences, and protease, transporter and protease/transporter sequences that may be used.

Table 8. Precursor polypeptide, rSAM, protease, transporter and protease/transporter sequences

Gene	Vector	Restriction Sites	Insert Sequence[a]
xncAB (Protein ID: WP_010848441.1, WP_010848442.1)	pET-28a(+)	NdeI_XhoI	AGCAAATTACAGCGTGAAATTGCAGCAAACAAAGCTCAACTGAGC CATGAAGACAAGAAGAAAACGCAGCACAAAGAGCTTGTTGACAG CCTGCTGGATACTGTCTCTGGTGGTTGGATAAACGCTTTTGGAAA CTGGGAGAGAGCCTTTCATTAAtacactgccgggggaggttttcttccccctt ctctttcttcattctggcgaataATGATAATGACGACATCAAAGAGTGAGA AGATCAAACATCTTGAGATCATTCTCAAAATTAGTGAACGATGCAA TATCAATTGCTCCTATTGCTATGTATTCAATATGGGTAACTCACTG GCTACCGATAGTCCTCCGGTCATATCGCTTGATAACGTGCTGGCG TTGAGGGGATTCTTTGAGCGCTCCGCAGCAGAAAACGAGATTGA AGTTATCCAAGTCGATTTTCACGGTGGTGAACCACTGATGATGAA AAAAGACCGTTTCGATCAAATGTGTGACATTCTTCGGCAGGGTGA CTATAGCGGTTCCCGGCTTGAATTAGCATTACAGACTAACGGTAT TCTGATTGATGATGAATGGATTTCACTGTTTGAAAAACATAAAGTC CATGCCAGCATATCAATCGATGGACCAAAACATATCAATGACCGC TATCGGTTGGACCGAAAAGGAAAAAGCACTTACGAAGGAACAATT CACGGCTTGCGCATGCTCCAGAATGCGTGGAAGCAAGGGCGACT CCCGGGAGAGCCCGGCATTCTCTCTGTGGCAAACCCCACAGCGA ATGGTGCAGAGATTTATCACCACTTTGCAAACGTCCTCAAATGTC AGCACTTCGATTTCCTCATACCCGACGCTCACCATGATGATGATAT TGATGGCATAGGTATTGGCAGATTCATGAATGAAGCGCTTGACGC ATGGTTTGCTGACGGTCGGTCAGAGATTTTTGTTCGAATCTTTAAC ACATACCTTGGCACGATGCTAAGTAACCAGTTTTACCGGGTTATT GGCATGAGCGCGAATGTAGAATCTGCTTATGCTTTCACGGTAACT GCCGACGGCCTGCTCCGTATTGATGATACTTTGCGTTCCACCTCT GATGAAATATTCAATGCCATTGGGCATCTCAGTGAATTGTCACTCT CCGGCGTACTCAATTCACCTAATGTCAAAGAATATCTTTCACTAAA TAGTGAACTGCCAAGTGATTGTGCAGATTGTGTGTGGAACAAAAT CTGTCACGGTGGCCGCTTGGTCAATCGCTTTTCACGGGCAAACCG TTTCAATAATAAAACCGTGTTCTGTTCATCAATGAGGCTTTTCCTT AGTCGCGCGGCTTCACACCTGATTACGGCTGGTATTGATGAAGAA ACAATAATGAAAAATATTCAGAAATAG (SEQ ID 194)

xncCDE (Protein ID: WP_013185693.1, WP_013185694.1, WP_013185695.1)	pCDFDuet-1	NdeI_XhoI	GAAAAAATCAATTTCTGGTTATCAAAGTTTTCATGTGCCGCCCTCG CTATTTGTTGTACATCTTGCCTTGCTGACTCGGGAAATTCGGTAAC ACTTAAGCTGAATTATGACAAATATTTCACGCCTCATGCAACTTTC ATCATTAATGGCCACCCGGTAAATATGATGATTGATACAGGTTCTT CGAAGGGCTTTTATCTTCAAGAGCCTCAACTAAAAAAAATACAAG GCCTCAAAAAAGAAAGCACTTATTACAGTACTAATATCACCGGGA AAAGACAGGAGAACACAGAGTATCTCGCCGCTTCTCTCGACATGA ATGGCCTTAAATTAAAAAACGTAACCGTGATCCCATTTAAACAATG GGGAGCGCTGATTTCTAACACAGGTAAATTGCCGGATGGCCCTGT TGTCGGTCTCGATGCGTTTAAAGATAAACAAATTATGCTGGATTTT GTGTCTCATTCATTCACGATGAGCGACAGTTTTATCCATAACATGC CGGTTCCGAAAGGCTTTAACGCATTCACTTTCCATATGTCTCCTGA TGGCATGGTTTTTGATGTTGATCAGTCTGGACACACATACCATTTG ATTCTGGACACCGGTGCCACTGCGTCTGTGATTTGGCGTGAAAGA CTTAAACAGTATGAACCCAAAAGCTGCCTGCTGGTCGATCCGAAG ATGGATAACGAAGGATGCCAGGCCACTCTGCTCACAATTAAATCA AAAACTGGAAATCCCCAGCATTTTGGTGCGGTTGTTGTTGTCGGA AATTTTAAACACATGGGCAACGTTGATGGCCTTTTAGGGAATAAC TTCCTCAGAAATCGAAAGGTACTTATAGACTTTAAAAACAAGAAG GTTTTTATTTCCGATGAGCACCGAAACAGAAAAGAATGACAACTC AATCTTTCGTGCCGAGGCTTTGCAACACAAACGAGAAGGTTGGCT CGGCGCTTCTCGTTTGCATATACCGTCAGCGCTCTCTATTTGTTGC CTGACAATCCTTGTTATTTTCTTTTTCATCATATTGATAATTGCATT TGGTTCGTACAGTGAACGGATAAATGTCATCGGAACCGTGGTTTA TAAGCCGCCTGCGGTATCACTGATTGCACAAAGCAGTGGAATCAT TACGCATTCACTGGCATTAGAGCAAACAAGAGTTAAGCGCAACGA GAGCATTTTTTCTATCAGTGGTGACACTCAGACAAATCTGGGTGC CACCAATGTTGAAACGGTAGAACTTTTAAATAAGCAACGTAACGC GCTGTCTAAAAAGCTTGATATTGCGGCCAATGAATCAAAAGCAAA CAAGATTTATCTCAGCGAAAAAATTAAAAATAAACAACAGGAAATA GAAAGTCTGCAAAACCTGATAGAAACTTCAGAAAAACAGCAAGCG TGGTTCGAGAAAAAATCAAACCTGTATGCGAATTTTAAGAAGAAA GGCATTGCGCTTGATGCTGAATGGATAAACAGAAAGAAAGATTAT TACGCATCCACATTAAGCATTTCTTCTGCAAAGGTCAAAGTGATAG CCCTGCTGGGAGAGTTGCAGGATCTGAAAAATGACGTTTCGGTTA TCGACAGGAAACTCGACAAAGAAACAGCATCTCTCACTGTCGAAA TAGCCGATATAGCACAAAAAATACTGATTACAGAAAAACAAAAAG AGTATTTAATCGTCGCGCCGTTTGATGGAATGATAACCAGTGTTA CAGCCCATATCGGTGAAAGAGTGACTGCCGGCCAGCAAATAGCC GTGCTGATACCACAAGGTGCGACAGAAAAGGTTGAGTTGTTTTCA CCGTCTGATTCTCTCGGTGAAGTGACCAGCGGACAGCAAGTCAG AATGAGAGTCTCGGCATACCCTTACCAGTGGTATGGAAAGATTGC AGGCATCATAGAAACGATATCGGCAGCACCGGTCAATGTCACCTC ACAGATGCAGATGAAAGGTGAAGAGGTAAAAAAGGGGCTTTTTC GGATTGTCGTACAACCAAAATTGACCGGACAACAAACAAACATTT CCCTTCTACCCGGCATGGAAGTGGAAACAGAGATCTATGTGAAAA CCCGAAAATTGTACGAATGGTTATTTATCCCCATTAAAGGGGCAT ATGAACGGGCGACAGACAGTACGGAATAAatATGCAGTATAAGAT GAGTGATTTTTTCGAGTTTTTCGTCAAAAAACTCCCGGTGATAATA CAAACAGAGACCACAGAATGCGGGTTGGCATGTCTGGCCATGAT TGCTGCCTGGTATGGCCGTGAGACTGATATCTACAGCATGAGAAA GGTTTTTGACGTGTCAAACAATGGCATGACATTAAGGCAGATCAT CACGGCGGCCGGGCGAATAAACATGAATACCAGAGCTGTGCGGC TGGAACTCAACGAACTCAGCAGTGTCAGGCTTCCGTGCATCTTGC

			ACTGGTCCTTTAATCATTTTGTCGTGTTAAAAAAATTCACAAAAAA AGGGGCAGTCATCCATGATCCCGCCTTGGGAAAAAGAACTGTCA CTCTGAAAGAACTCTCAAATAAGTTTACGGGCATCGCTCTGGAAG TCTGGCCCCAGACGGAGTTTAAAAAGGAAAAGGTCAGTGAAAGC ATAACCATCACGGATATGTTTCGCGGTGTTGCCGGCCTTAAGAAT ACGCTGTTTAAAATCATTCTGTTGTCGCTCTTTATTGAAGTACTGG CACTTTCCATCCCTCTCAGCTCTCAATTCATTATTGATGTTGTTCTA CGGTCCAGTGACCTCAGTATGCTGAATTTCATTGTCATTGGAATC GTTCTTCTGCTCTCCCTGCGCGCTGCTTTCAGTATTGTGCGCGCC TGGGCTCTTATGGCAATGCGTTACTCACTTGGCATACAGTGGAGT TCCGGTTTTTTTAACCGGTTACTCAGATTGCCGGTCACTTTTTTTG AAAAACGTCACGTAGGTGATATCGCCTCCAGATTGACATCGTTGA GCGAAGTTCAAGAAGCCTTTACAGCAGAAATGCTGACTTCGTTAC TTGATGTACTTATTCTCATAACGCTGGCTGTGCTCATGTTCTGTTA CAGCCCTCTTCTGACCCTTCTCCCGCTACTCATGACTACCGTTTAT CTTGGGGTCAAATTTGCTTTTTATGACAGATACATGGGAGCAAAA GTAGAAGCAATTACGCATGAAGCGCAGCAATCATCCTACTTTCTC GAAACAATACGAGGCGTAGCGTGCGTGAAAGTATTTGGCCTGAC AGAATTCCGACGTATCACATGGCTTAACCGGGTGATTGATACTGC CAATGCCCGGGCCCATTTATTTAAGATAGACCTCATCAGCCAAAC GCTTTCAGGTTTCCTGACGGGGCTATCATCGGCGGCCATTTTGTT TATGGGGAGTCATCTCACAGAACGCGGCCTGATCACTGCCGGCA TTCTGTTTGCTTTTCTGCTCTATACCGATATGTTTCTGACACGTTCA GTGAAGGTAATAAATTCACTGTTTGCTTTTCGCCTTATTTCGATAC ACACGCACCGATTGACCGATATTGCAACAGCCCAGACAGAAAATG CATGGAACCCGGAAGATCCCGTCACACTCGATAATGTAAAAGGCC GGATAACACTGAACAATCTCACATATCGGTACGGAGAAACTGAAC CCTGTATTTTCGACTGTATCGACATGGAAATTAATGCTGGTGAGA GTGTGGCGATCGTAGGTCCGTCAGGTTGCGGTAAATCGACACTT CTCCGGGTCATGGCCGGCCTGGTTCTCCCTCAGTCAGGCGATGT GTCAATTGATGATGTCAGTGTGAAAAAAATGGGTATTGACGAATA TCGCAGACACACGGCGTTTGTCATGCAAGATGATAAGCTTTTTGC TGCCTCATTGATGGATAACATATCCGCTTTTGATCCACAGCCAAAT ATTGATTGGATACATGAATGCGCTAAGGCGGCGGCAATACACGAT GAAATTATGACTATGCCGATGCAGTACGAAACCATGGTGGGTGAC ATGGGGAGCATTCTTTCAGGCGGACAAAAACAGCGTGTATCCCTT GCACGGGCACTTTACAAGTGTCCGCGTATCCTCTTTCTTGATGAG GCCACCAGCCATCTCGACGTTTTTAATGAACGCAAGATAAATGAG GCTGTAAAGCAGATGCCGATTACGCGTGTATTTGTGGCTCATCGG CCAGAAATGATCGCTGTCGCAGACCGAGTTTATAACCTGAGGGAT AAGACCTTTACAACGTAA (SEQ ID 195)

xncBCDE	pCDFDuet-1	NdeI_XhoI	ATGACGACATCAAAGAGTGAGAAGATCAAACATCTTGAGATCATT CTCAAAATTAGTGAACGATGCAATATCAATTGCTCCTATTGCTATG TATTCAATATGGGTAACTCACTGGCTACCGATAGTCCTCCGGTCA TATCGCTTGATAACGTGCTGGCGTTGAGGGGATTCTTTGAGCGCT CCGCAGCAGAAAACGAGATTGAAGTTATCCAAGTCGATTTTCACG GTGGTGAACCACTGATGATGAAAAAAGACCGTTTCGATCAAATGT GTGACATTCTTCGGCAGGGTGACTATAGCGGTTCCCGGCTTGAAT TAGCATTACAGACTAACGGTATTCTGATTGATGATGAATGGATTTC ACTGTTTGAAAAACATAAAGTCCATGCCAGCATATCAATCGATGG ACCAAAACATATCAATGACCGCTATCGGTTGGACCGAAAAGGAAA AAGCACTTACGAAGGAACAATTCACGGCTTGCGCATGCTCCAGAA TGCGTGGAAGCAAGGGCGACTCCCGGGAGAGCCCGGCATTCTCT CTGTGGCAAACCCCACAGCGAATGGTGCAGAGATTTATCACCACT TTGCAAACGTCCTCAAATGTCAGCACTTCGATTTCCTCATACCCGA CGCTCACCATGATGATGATATTGATGGCATAGGTATTGGCAGATT CATGAATGAAGCGCTTGACGCATGGTTTGCTGACGGTCGGTCAG AGATTTTTGTTCGAATCTTTAACACATACCTTGGCACGATGCTAAG TAACCAGTTTTACCGGGTTATTGGCATGAGCGCGAATGTAGAATC TGCTTATGCTTTCACGGTAACTGCCGACGGCCTGCTCCGTATTGA TGATACTTTGCGTTCCACCTCTGATGAAATATTCAATGCCATTGGG CATCTCAGTGAATTGTCACTCTCCGGCGTACTCAATTCACCTAATG TCAAAGAATATCTTTCACTAAATAGTGAACTGCCAAGTGATTGTGC AGATTGTGTGTGGAACAAAATCTGTCACGGTGGCCGCTTGGTCAA TCGCTTTTCACGGGCAAACCGTTTCAATAATAAAACCGTGTTCTGT TCATCAATGAGGCTTTTCCTTAGTCGCGCGGCTTCACACCTGATTA CGGCTGGTATTGATGAAGAAACAATAATGAAAAATATTCAGAAAT AGtggagccggacaATGGAAAAAATCAATTTCTGGTTATCAAAGTTTT CATGTGCCGCCCTCGCTATTTGTTGTACATCTTGCCTTGCTGACTC GGGAAATTCGGTAACACTTAAGCTGAATTATGACAAATATTTCAC GCCTCATGCAACTTTCATCATTAATGGCCACCCGGTAAATATGAT GATTGATACAGGTTCTTCGAAGGGCTTTTATCTTCAAGAGCCTCA ACTAAAAAAAAATACAAGGCCTCAAAAAAGAAAGCACTTATTACAG TACTAATATCACCGGGAAAAGACAGGAGAACACAGAGTATCTCGC CGCTTCTCTCGACATGAATGGCCTTAAATTAAAAAACGTAACCGT GATCCCATTTAAACAATGGGGAGCGCTGATTTCTAACACAGGTAA ATTGCCGGATGGCCCTGTTGTCGGTCTCGATGCGTTTAAAGATAA ACAAATTATGCTGGATTTTGTGTCTCATTCATTCACGATGAGCGAC AGTTTTATCCATAACATGCCGGTTCCGAAAGGCTTT 33 AACGCATTCACTTTCCATATGTCTCCTGATGGCATGGTTTTTGATG TTGATCAGTCTGGACACACATACCATTTGATTCTGGACACCGGTG CCACTGCGTCTGTGATTTGGCGTGAAAGACTTAAACAGTATGAAC CCAAAAGCTGCCTGCTGGTCGATCCGAAGATGGATAACGAAGGA TGCCAGGCCACTCTGCTCACAATTAAATCAAAAACTGGAAATCCC CAGCATTTTGGTGCGGTTGTTGTTGTCGGAAATTTTAAACACATG GGCAACGTTGATGGCCTTTTAGGGAATAACTTCCTCAGAAATCGA AAGGTACTTATAGACTTTAAAAACAAGAAGGTTTTTATTTCCGATG AGCACCGAAACAGAAAAGAATGACAACTCAATCTTTCGTGCCGAG GCTTTGCAACACAAACGAGAAGGTTGGCTCGGCGCTTCTCGTTTG CATATACCGTCAGCGCTCTCTATTTGTTGCCTGACAATCCTTGTTA TTTTCTTTTTCATCATATTGATAATTGCATTTGGTTCGTACAGTGAA CGGATAAATGTCATCGGAACCGTGGTTTATAAGCCGCCTGCGGTA TCACTGATTGCACAAAGCAGTGGAATCATTACGCATTCACTGGCA TTAGAGCAAACAAGAGTTAAGCGCAACGAGAGCATTTTTTCTATC

			AGTGGTGACACTCAGACAAATCTGGGTGCCACCAATGTTGAAACG GTAGAACTTTTAAATAAGCAACGTAACGCGCTGTCTAAAAAGCTT GATATTGCGGCCAATGAATCAAAAGCAAACAAGATTTATCTCAGC GAAAAAATTAAAAATAAACAACAGGAAATAGAAAGTCTGCAAAAC CTGATAGAAACTTCAGAAAAACAGCAAGCGTGGTTCGAGAAAAAA TCAAACCTGTATGCGAATTTTAAGAAGAAAGGCATTGCGCTTGAT GCTGAATGGATAAACAGAAAGAAAGATTATTACGCATCCACATTA AGCATTTCTTCTGCAAAGGTCAAAGTGATAGCCCTGCTGGGAGAG TTGCAGGATCTGAAAAATGACGTTTCGGTTATCGACAGGAAACTC GACAAAGAAACAGCATCTCTCACTGTCGAAATAGCCGATATAGCA CAAAAAATACTGATTACAGAAAAACAAAAAGAGTATTTAATCGTCG CGCCGTTTGATGGAATGATAACCAGTGTTACAGCCCATATCGGTG AAAGAGTGACTGCCGGCCAGCAAATAGCCGTGCTGATACCACAA GGTGCGACAGAAAAGGTTGAGTTGTTTTCACCGTCTGATTCTCTC GGTGAAGTGACCAGCGGACAGCAAGTCAGAATGAGAGTCTCGGC ATACCCTTACCAGTGGTATGGAAAGATTGCAGGCATCATAGAAAC GATATCGGCAGCACCGGTCAATGTCACCTCACAGATGCAGATGAA AGGTGAAGAGGTAAAAAAGGGGCTTTTTCGGATTGTCGTACAACC AAAATTGACCGGACAACAAACAAACATTTCCCTTCTACCCGGCAT GGAAGTGGAAACAGAGATCTATGTGAAAACCCGAAAATTGTACGA ATGGTTATTTATCCCCATTAAAGGGGCATATGAACGGGCGACAGA CAGTACGGAATAAatATGCAGTATAAGATGAGTGATTTTTTCGAGT TTTTCGTCAAAAAACTCCCGGTGATAATACAAACAGAGACCACAG AATGCGGGTTGGCATGTCTGGCCATGATTGCTGCCTGGTATGGC CGTGAGACTGATATCTACAGCATGAGAAAGGTTTTTGACGTGTCA AACAATGGCATGACATTAAGGCAGATCATCACGGCGGCCGGGCG AATAAACATGAATACCAGAGCTGTGCGGCTGGAACTCAACGAACT CAGCAGTGTCAGGCTTCCGTGCATCTTGCACTGGTCCTTTAATCA TTTTGTCGTGTTAAAAAAATTCACAAAAAAAGGGGCAGTCATCCAT GATCCCGCCTTGGGAAAAAGAACTGTCACTCTGAAAGAACTCTCA AATAAGTTTACGGGCATCGCTCTGGAAGTCTGGCCCCAGACGGA GTTTAAAAAGGAAAAGGTCAGTGAAAGCATAACCATCACGGATAT GTTTCGCGGTGTTGCCGGCCTTAAGAATACGCTGTTTAAAATCAT TCTGTTGTCGCTCTTTATTGAAGTACTGGCACTTTCCATCCCTCTC AGCTCTCAATTCATTATTGATGTTGTTCTACGGTCCAGTGACCTCA GTATGCTGAATTTCATTGTCATTGGAATCGTTCTTCTGCTCTCCCT GCGCGCTGCTTTCAGTATTGTGCGCGCCTGGGCTCTTATGGCAAT GCGTTACTCACTTGGCATACAGTGGAGTTCCGGTTTTTTTAACCG GTTACTCAGATTGCCGGTCACTTTTTTTGAAAAACGTCACGTAGGT GATATCGCCTCCAGATTGACATCGTTGAGCGAAGTTCAAGAAGCC TTTACAGCAGAAATGCTGACTTCGTTACTTGA
			34 TGTACTTATTCTCATAACGCTGGCTGTGCTCATGTTCTGTTACAGC CCTCTTCTGACCCTTCTCCCGCTACTCATGACTACCGTTTATCTTG GGGTCAAATTTGCTTTTTATGACAGATACATGGGAGCAAAAGTAG AAGCAATTACGCATGAAGCGCAGCAATCATCCTACTTTCTCGAAA CAATACGAGGCGTAGCGTGCGTGAAAGTATTTGGCCTGACAGAA TTCCGACGTATCACATGGCTTAACCGGGTGATTGATACTGCCAAT GCCCGGGCCCATTTATTTAAGATAGACCTCATCAGCCAAACGCTT TCAGGTTTCCTGACGGGGCTATCATCGGCGGCCATTTTGTTTATG GGGAGTCATCTCACAGAACGCGGCCTGATCACTGCCGGCATTCT GTTTGCTTTTCTGCTCTATACCGATATGTTTCTGACACGTTCAGTG AAGGTAATAAATTCACTGTTTGCTTTTCGCCTTATTTCGATACACA CGCACCGATTGACCGATATTGCAACAGCCCAGACAGAAAATGCAT

			GGAACCCGGAAGATCCCGTCACACTCGATAATGTAAAAGGCCGG ATAACACTGAACAATCTCACATATCGGTACGGAGAAACTGAACCC TGTATTTTCGACTGTATCGACATGGAAATTAATGCTGGTGAGAGT GTGGCGATCGTAGGTCCGTCAGGTTGCGGTAAATCGACACTTCTC CGGGTCATGGCCGGCCTGGTTCTCCCTCAGTCAGGCGATGTGTC AATTGATGATGTCAGTGTGAAAAAAATGGGTATTGACGAATATCG CAGACACACGGCGTTTGTCATGCAAGATGATAAGCTTTTTGCTGC CTCATTGATGGATAACATATCCGCTTTTGATCCACAGCCAAATATT GATTGGATACATGAATGCGCTAAGGCGGCGGCAATACACGATGA AATTATGACTATGCCGATGCAGTACGAAACCATGGTGGGTGACAT GGGGAGCATTCTTTCAGGCGGACAAAAACAGCGTGTATCCCTTGC ACGGGCACTTTACAAGTGTCCGCGTATCCTCTTTCTTGATGAGGC CACCAGCCATCTCGACGTTTTTAATGAACGCAAGATAAATGAGGC TGTAAAGCAGATGCCGATTACGCGTGTATTTGTGGCTCATCGGCC AGAAATGATCGCTGTCGCAGACCGAGTTTATAACCTGAGGGATAA GACCTTTACAACGTAA (SEQ ID 196)
smcAB (Protein ID: WP_071845309.1, WP_047728930.1)	pET-28a(+)	NdeI_XhoI	TCTAAATTAGCCAAAGAAATTAACATGAATAAAGCAGCCGTCACC GTTGCAGCTGATAAAAAAGACGCACGAAAAGCACTGGCTCAATCT ATGCTGGATAGCGTTTCTGGCGGTTGGGTCAACGCCTTTGCGCGT TGGTCCAAAAGCTTCTAAttgaccttggtgcagggtgggagaccgccctgcac tttctcctttgttgaacagtggtacgggcaATGACGAATAAGAAAAAAATAAA GCATCTTGAAATAATTTTAAAGGTTAGTGAACGATGCAACATTAAC TGCACGTATTGCTATGTATTCAACCTGGGCAATGATTTGGCAATA AATTCAAAACCAATTATTTCTCATAAAATCATTGAAGATTTGAGAG GTTTTTTCGAGCGGGCCTGCCAGGAGTATGAAATAGAAACGGTTC AGGTTGACTTTCATGGCGGCGAACCGTTAATGATGGGGAAAGAG CGTTTCGACAATGCCTGCAAAGAGCTTATCTCAGGTGACTATAAT GGCGCCAGGCTCAACCTTGCCTGTCAGACAAACGCTATCCTTATT GATAATGAGTGGATTGATATTTTCTCGAAATATAATATCAGCGTGG GGATTTCTATTGATGGCCCCAAGCACATTAACGACAGGCACCGCC TGGATAGAAAGGGACGCAGCACCTACGAAGGTACGGTAAAAGGG CTGGAGATGCTGCAGGTTGCCTGGAAAGCGGGCCGATTGATCGA TGAACCCGGCATCCTGTGCGTCGCCAATCCTTCGGTAAAAGGCG CTGAAATCTATCGTCATTTTGTCGATGTACTGAAATGCAAAAAATT TGATTTCCTCATTCCGGATGAAAGCCATGACACCTGCACGGATCC GGACGGACTGGCGGATTTTTATTGCTCGGCGCTGGACGAGTTCTT TTTGGACGCGGATAAAGAGGTGTATGTGCGCTACTTCCATACGCA CATCCAATCCATGTTGAGTTCAGAATTCAATCCGGTAATGGGAGT AAGCAAAGCCGGGAACGATACTCTCGCTTTCACGGTGAGTTCCGA TGGTGAACTGTATGTGGATGATACGCTGAGAGCAACCAATGACCC TATATTTACGCCTATTGGTAATATTCAACATTTAATACTGTCAGAC ACTCTCGCCTCATGGCAGATGACAAAGTATATGGCTGTGAATAGT CAGCTTCCTACCGTTTGCGGTGACTGTGTCTGGCAAAAAGTTTGT GGCGGAGGGCGTCATATTCAGCGTTATTCTACAGCCGATGATTTT AACCGTGAAACCGTTTTTTGTCCGTCGGTAAGAAAGATCATGAGC CGTGCGGCTTCGCATTTGATTGAATCGGGCGTGGCAGAGGATAT AATCATGAAAAACTTAGAGGTTAACTCATGA (SEQ ID 197)

smcCDE (Protein ID: WP_047728928.1, WP_080490739.1, WP_047728923.1)	pCDFDuet-1	NdeI_XhoI	ATCAAGCGGCTATCCTTATTGGCGTTCTTGTTTTCCGGCATCAGC ATGGCGAGTCTTCCCGCTGATTTTGGGCGGTTGCGGTATGATGAA CGTGGACTGCCGTTAATTGATGTCCGGATCGATAATCGTCTTCAT ACCTTAATGTTGGATACCGGCAGCGGGGAGGGGATGCATCTTTAT AAACACGATCTTGACAACTTAGTGGCTAATCCTGGCCTGCAGGCG ACCGAACAAGCCCCTCGCCGGTTGATGGATGTTTCAGGGGGTGA AAATAAAGTTTCCTCATGGAAGATTAATCGATTACTTATTTCCAAT ATTCCTTTCGATAATGTTGAAGCGGTAAGTTTTAAACCATGGGGA TTAAGCATCGGCGGTGATGTCCCTATGAATGAAGTGATGGGGTTG GGGCTTTTTCGAGAACGCAGAGTGCTGATGGATTTTAAAAACGAT CGGTTAAAAATATTGGCCGACTTGCCATCTGACATAAAGAAATGG TCATCGTACCCCATCGAACCAACCGCATCGGGATTGCGCGTTACC GCCTCCGCAGGCGGTATGCCTTTGCATTTGATTGTCGATACTGCG GCCAGCCATTCTCTGCTGTTTTCAGACCGTTTGCCGCCGGGCCTC CTTTTCTCTGGGTGCCGCGACATTGAGCCGGAAGCGTCGAATCTG GATTGCCGGGTGACAAAAATCGCTTTTACGGATCGCGAAGGTAA GGCTCGTGATGACCAGGCCGTCGTTGCCTCTGGTGCCACGCCCC CGGAACTGGATTTTGACGGTCTTTTGGGGATGAAGTTTATGCGGG GACATCAGGTGATCATCGATATGCCTGAACGCCTGCTCTATATCA GCCGTTAGcgtgATGGACAAAGAAAACTCGTTTTTCCGCCAGGAG GCGTTGCAGCATAAAAAAAATGCCTGGCTGGGCGATTTTACCGTT TCGGCGCCATCAGTGTTGCCCATCGCGTTATGGAGCGCCGTTGG CGTTTTGCTGTTGGCTACCCTTCTGTTATTCACCACTTATGCCAAA AGAGTCCCCGTGACCGGGCGAGTCATCTATACGCCTTCCGCTGCT GAGGCGGTGTTTAACCATGACGGGATTATCGGCCGCATCGAAGT GCACCAAGGGGAAAGGGTTAAGAAAGGGGATGTCATCGCGACGT TTTCACGCGATGTCGCCTATGTCGGGGGAGGCATGAATCAGGCA TTGCAAGATGCGGCGCAGCGCCAGCTTACCGAGTTGCAAAAGCG CGCGGGAGAGCGGCGTAAAGAGGGAGAAGAAGAGCGCTTGCGT TTACGTGAGAAAGTCAGCGCCAAAGAACGGGAAATGGTGGCGAT TCAAGCTGCGGCCGAAGCCGAATCGGAGCACATCGTCGGTTTGA AGAAGCGGATGGCGCTTTATCAACAGCTGTTACTGAAAGGTATTA CGACCGTACAAGAGAAAATTGAGCGGGAGAACGAATATCATAATT CTATTGCACAGCTGAACACGCATCGAATCAATATCGCGCGGGTGA AAGGAGAGCTGCTGCAATTCGAGGATGAGCTGGCTCGCTCTGAA TCGCAAGAAAAACAGTCTATTACTGACATTCAACAGCAGAAGGTC ACGCTGCAACAGCAGGTGATTAATGCCTCTGCGGTCGTGGAGTC TCGGGTTGTGGCTCCGCTTGATGGCGTCGTCGCTTCAATGAGCAT TTTGGAAGGACAGAGAGTGACCGCCGGCGCAGTTGCCGCAGTGG TGGTGCCGGAAAATGCACGTCCGTTCGTTGAAATGTGGATCCCG CCCTCTGCGCTGCAGGAGGTGAAAGCGGGTCAGCATGTTTTCAT GCGCGTCGCATCCTTGCCGTGGGAGTGGTTTGGGAAAGTGTCCG GCACGGTTGCCGCCGTCAGCGAGAGTCCTGAGGCGCTGACGGG AAATAATCGACGTTTTCGCGTGCTGATCGCGCCCGATGTCGGAAC GCGAGCGCTGCCTGCGGGAGTGGACGTTGAGGCCGACATATTGA CGACGCATCGGCGCATCTGGGAATGGCTCTTCTTACCATTAAAAC AAAGTATTAACCGCATGACGGCTGAGAGTTGAcacATGCTTTTTTC CTGGCAAAAAACACCGCTGATTCTACAGTCGGAAACGAATGAGTG TGGGTTGGCCTGTTTGGCCATGATGGCCGGTTATTTCGGCAAACG CATCGATCTTGCTTCGGCGCGTACCCTTCACGGGATCGGCAGCCA CGGGATGACGCTGCGAGATCTCATTACGGCGTTTGAACGTGTGG GGATGACGGCTCGTGCTTCGCGCGTAGAGCTGGATGAACTGCGT TCTCTCAGCCGCCCTGCGATTCTTCACTGGTCATTCAATCATTTCG TGGTGCTGGTGAAAGTGACGCGTCGGGGCGCGGTGATCCTGGAT

			CCTGCCATTGGTCGCCGCAGCATTTCATTGCGTGAACTGTCGGAT AAATTTACCGGCGTTTTGGTGGAAGCATGGCCTGCGGAGACCTTC GATAAGAAAGCGCTGGAAATGAATGTCACCGTATCCGATCTTTTT CGTGGCGTACGGGGCTTAAGACGCATTTTTACCGGCGTTCTGATG CTTTCGGTCTTGGTGGAACTGCTCTCCATTGCGGTACCCGCCGCG TCACAATTTACTATCGATACGTTAGTGCGTTCATCAGACCGCGAA GGAATATTTTTTGTCGGTATCGTGGTCATTTCCGCATTGCTGATTA AGTCCGCCTTTTCGGTGGTGCGTGCCTGGATTTTGATGAATCTGC GCTATACGCTCGGCGTGAAATGGGCTGAAATGTTCTTTAACCGGC TTATCAAACTTACGCTGTCATTTTTTGAGAAGCGGCACACCGGCG ATATCGCGTCGCGCTTCCAGTCGTTGACCGCCATTCAGGAAGCGT TTACGGCCGATATGGTTGCCTCTCTCTTGGATGCGATTGTGATTG TCATTTCAATGGCGATCATTTTTACCTATTCACCTGTGCTGGCCAT CGGCCCCCTGATCGCCGCCTGCGCCTATGCCGCCTTGAAGGCGG GCCTGTTCTCGACCTACCGCAATCGTAAAATTGAACATATCGCCTT CGAAGCGGTGCAATCCTCCCACTTCCTTGAAACCGTCAGAGCGAT CGGCGCGATCAAAATGTTGAACCTGACGCCGGTTCGTCGGCGCG AATGGGTCAACCATGTGGTCAACAGCACGCATGCGGGGAACCAG CTGTTTAAACTCGATCTGCTGACCAACACGGCGGCCGTGCTGCTG GTGGGATTTTCCGGGATTTTCGTGCTTAGCGTCGGGGCCATCGG ATTTGATAAAGGCATTACGACTGGCGCCTTGCTGGCCGTGATGCT GTATGCCGATATGGTGATTACCCGCACGGTGAAGTTAGTCAATGC GGTTTCTGATTTTTGCCTGGTATCCATGCACAGTCAGCGTTTGACT GACGTGGCTGTTTCACCCGTGGAACGGGATGAGGGAGAACAAGT GTCGCCACAGCTGAATGGGCATATCGTGATCCGCAACTTAGCGTT CCGCCATTCCCAGACCGAACGCAACATCTTCGAGGGGATCAATCT TGAGATCATGCCAGGGGAAAACGTCGCGATCGTCGGGCCGTCCG GGTGTGGTAAGTCAACATTCCTCCATGTGCTGGCGGGGTTGTAC GAATCTACCGAAGGGGATGTTTTCATTAACAACGTGGGGATGTCT GGCATGGGCAAACGAGACATTCGTGAACATGTCGCTTTTGTCATG CAGGACGACAAACTCTTGGCTGGAACCATACAGCAGAATATTACC GGTTTTACCGCGTCCCCCGATGTGGAACGCATGGCTGAATGCGC CAATCATGCCGCGATTGACGAAGAAATCAGCGCATTTCCACAGGG ATATGAGTCGATGATCGGTGATATTGGTAGCACGCTTTCTGGCGG GCAACGCCAGCGTATTTCTATCGCCAGAGCGCTATACCGGCAACC TCGTGTGCTGCTGCTTGATGAGGCAACCAGCGATCTTGATATCGA TAACGAGAAAAAGATCACTCGCGCCATCGGGCAATTGCCGATAAC CCGCATTTTTGTTGCTCATCGCCCAGAAATGATCAAGTCAGCGGA TCGGGTCTTTAATCTTCATCTGAATGCCTGGGTGAAGCAGGAAAA TCGGGGGGGCGCTACAATGTTGATCGCCGACAAGGTTCACATAA GCTGA (SEQ ID 198)

etcAB (Protein ID: WP_017801003.1, WP_017801004.1)	pET-28a(+)	NdeI_XhoI	AGCAAATTACAGCATGAAATCGCGTCAAACAAAGCCCGCCTGAAT AATGCTGACGATAAAAAAGCACAGCGTAAAATCCTTGTTGATAGC CTGCTGGATACTGTCTCTGGCGGCTGGATAAATGCCTTTGCTAAC TGGACTAAGCGTATCTAAttgagactgcacgggggagatttccacccccgtgt tttcccatggaggaggatacacATGACACAGTTAAAAGGCGAAAAAATAA AGCATCTTGAAATAATTTTAAAAATTAGTGAACGCTGCAATATTAA TTGTACTTACTGCTATGTATTCAATATGGGTAATACACTGGCAACC GATAGCACGCCGGTAATTTCTCTGGATAACGTATACGCGCTGAGG GGATTTTTTGAACGATCGGCTGCCGAAAATGACATTGAGGTTATT CAGGTAGACTTTCACGGTGGCGAACCGCTGATGATGAAAAAAGA CCGTTTCGATCGCATGTGCCAGATTCTCTTGCAGGGTAACTACCG CAGTTCAAAATTTGAACTGGCATTACAAACCAATGGCATTTTGATT GATGACGAGTGGATTGCGCTTTTTGAAAAACATCAGGTGCATGCC AGTATATCGGTCGACGGACCAAAACATATCAATGACCGTCATCGG TTAGACCGTAAGGGGAAGAGCACTTACGAGGGCACAATTACCGG TTTACGCCTGCTGCAAAATGCGTGGCAGCAAGGGCGTCTGCCAG GTGAACCAGGCATACTTTCAGTGGCCAACGCCAATGCAAATGGTG CGGAGATTTATCGCCACTTTGCCGATACTCTCCAGTGCCAGCGTT TCGATTTTCTTATACCAGACGATCATCACGACGATAGCCCTGATG GCGAAGGTGTAGGCCGATTTCTGAACGAGGCACTGGATGCATGG TTTGCTGATGGGCGGCCAGAAATCTTTATTCGAATCTTTAATACTT ATCTCGGCACCATGCTAAACAGCCAGTTTAATCGGGTGCTTGGTA TGAGTGCTAATGTTGAGTCCGCCTATGCCTTTACAGTAACAGCCG ACGGCATGCTGCGTATTGATGACACATTGCGTTCGACATCTGATG AGATATTCAATGCCGTTGGGCATGTCAGTGAATTATCGCTGGCGA GGGTACTTGAAACATCTTGTGTTAAAGAATATCTCGCGTTAAGCA GCAATCTGCCGACAGTGTGCGCAGAATGCGTATGGAATAATATCT GCCACGGCGGCCGTCTGGTAAATCGTTTTTCACGCACTAATCGTT TCAACAATAAAACCGTTTTCTGCAAATCGATGAGATTATTTCTTAG TCGCGCTGCATCGCATCTTATGGCATCGGGCGTGGATGAAAAAG AAATCATGAAAAACATTCAAAAATAG (SEQ ID 199)

etcCDE (Protein ID: WP_017801005.1, WP_017801006.1, WP_026111678.1)	pCDFDuet-1	NdeI_XhoI	AAGATGATAATAACCTGGTTATTAAACCGCTTATATTTTGTATTCG CCTTTAGCACGACACTATCCTTTGCTGATATGGAAAAATCCGTAAC CTTAACGCTGAGCTTTGATCAGCTTGCCACCCCGCATGCAAATTT CGTCATCAATGGCACCCCGGTCTATGCCATGGTTGATACGGGTTC TTCATTTGGTTTCCATCTTTATCAAAATCAACTTAATAAAATCAAAG GATTAAAAAAAGAACGTACATATCGTAGTACTGATGGAAAAGGTA AAGTTCAGGAAAATATAGCGTATCTGGCTAAATCTCTCGATATGA ATGGGTTGAAATTAAGAGATGTCCCCGTCACTCCATTTAAGCAGT GGGGGCTGATGATCTCTGGCGAAGGTGAATTGCCGCAGAGCCAG GTCGTGGGGTTAGGTGCATTTAAAGATAAACAAATATTACTGGAT TATAAGGGGAAATCACTCACCATTGGCGACAACATCGCTTCTGAA TCGCAAATCAAAGAAAATTTTCAGGAATATTCTTTTCAAATGTCTT CCGATGGCATGATCTTTCAAGCCGAGCAATCCGGGCATAAGTATC ATCTGATTATGGATACAGGTTCCACCGTTTCCATAATCTGGCGTG AGAGACTTAAATCCAGACAACCTGAGAGCTGTCTTATTGTCGATC CTGAGATGGATAATGAAGGATGCGAGGCACTGATGCTGGAAACG AAATCGAAGAATGGCAAAATCGAGCATTTTGGCGCGGTCATTGTA GCCGGTGACTTTGAACATATGGGCAATATTGATGGACTTATAGGT AACAACTTCCTCAAAAGCAGAAAGCTATTGATAGATTTTAAAAATA ATAAGGTTTTTATTTCCGATGACAACAGAAAAGGATGATGAGTCA GTCTTTCGTGCCGAGGCATTGCAACATAAGCGTGAGGGATGGTTT GGCCCTTCCCGTCTGCATGTCCCGTCAGGTCTCACTATTTTTCTGA TAACCGGCCTGATAACCGGCATTTTCACTGTATCCATTATTACGTT TGGTTCGTACAGCGAACGGATAAACGTCACCGGAATGGTGGCTT ATGATCCTCCAGCGGTGGCGTTAATGGCACTACGTGATGGGATAA TAACCCGTTCCTCTGCATTTGAGGGAACAATCATAAAACGCGGCC AGCTGGTTTTCACGGTAAGCAGTGATATTCATACCAACCTTGGCC CTGCCAACGTTGAAATGATGGCGCTGTTAAAAAAGCAACGTGATG CACTGTCTAAAAAGCTTGAGATCACCATTAGCAATGCTCAAAAAA ATAGTCTCTATCTGGCCAGTAAAACTAAAATAAAACAGCAGGAAA TTAACAGCCTGGAAGCGTTGATACAAGAAAGCGAAATTCAGAAGG AATGGTTCGCAGAAAAATCCAGGCTGTATACCCACTTAAGAAAAA AAGGCATCGCGCTTGATTCGGATCTGATAGACAGGCGAAAAGATT ATTATTTATCAGCAGAAAGTTTATCTTCATCGAAGGTAAGGCGGAT CACTCTGCAAGGTGAGTTGCTGGAGTTACAGAAACAAGCGTCATC TGTAGACAGGGATTTAAATGAAAAAAAAGAATCCTTTATTATAGAA CTGGCAACCATTGATCAAAGGATTCTTGATGCTGAGAAAAACAAA GAATATTTAATTGTCGCCCCCTTTGATGGCGTCATAACCAGCGTA AGCGCACATATTGGTGAAAGGGTAACAGCTGGACAGAGAATAGC TGTGCTTGTGCCGCAAGGCGCAACGGCAAAAGTTGAGCTACTTTC GCCTTCTGATTCAATTGGTGAAGTCGTCAGAGGGTTGCAAGTAAA AATGAGAGTGGCCGCATACCCTTATCAGTGGTATGGGAAAATCCG TGGCGCGATAGAAGCGATATCGGTAGCACCAGTCAATATGACATC CCCGGCACAGGCAAAGAGTGATTATAGCGGCAAAGGACTTTTTC GCATCATTGTCACACCAGAGCTGACAGAGCAGCAATTGAATATTT CGCTTTTACCTGGCATGGAGGTCGAAGCGGAAATATATGTTAAAA CCAGAAAAGTTTACCAATGGTTATTTATACCTGTCAGGCGGGCAT ATGAACGTGCAACGGACAGCATGGAATAGagATGCAATATAATAT CAGCGCATTTTTTCAGTCTTTTAGCAAAAGGCTACCGGTAATAATG CAAACAGAGGTTACTGAGTGCGGATTAGCTTGCCTGGCAATGATA GCCGCATGGTATGGTCGCAAGACAGATATTTACGGGATGCGAAA ACTTTTTGACGTCTCAAGTAACGGCATGACATTAAGGCAAATAAT GACAGCCGCAGGACGAATAAACCTGAATGCCCGTGCAGTGCGGC TTGAGCTGGAGGAGCTGAGCAGCACTAAACTTCCGTGTATTTTGC

			ACTGGTCATTCAACCATTTCGTGGTGTTGAAAAAGATAAGCAAAA AAGGCGCTATCATCCATGACCCCGCATCCGGAAAGAGAATTATCA GCATCAATGAACTGTCCAATAAATTTACCGGCATCGCTCTGGAAG TGTGGCCTCAGGCCGAATTTAAAAAAGAAAAAATCAGCGAGAGTA TTACTGTCAGCGATATGTTTCGCGGCGTAGACGGACTTGGGCGT GTGCTGTGTAAAATTCTTCTGTTATCACTGTTTATCGAGATTCTGG CCCTTTCTGTTCCTCTTGCCTCTCAATTTATTATTGATATTGCGTTA AAGGCAAGCGACCTCAACATGTTGAATTTTATTATAACTGGCGTC GTTTTTCTGCTTATCCTGCGTGCGATTCTTAGTATGGTTCGCGCCT GGACGCTTATGGCGATACGTTATTCACTTGGCATCCAGTGGAGCG CCGGATTTTTTAACCGCCTGCTAAAGCTGCCGGTGGCCTTTTTTG AAAAGCGCCATGTCGGAGATATTGCCTCGAGGCTGACTTCGCTAA ATGAGGTGCAGGAAGCATTTACGGCAGAAATGCTTACTTCTCTGC TCGACGTACTTATTCTGCTGGCGCTGATCGCGCTGATGTTCGCTT ACAGCCCATTTTTGGCCATCATATCCCTGCTGATGGCCGCTGTTT ATCTGGGGGGTGAAATTAATGTTCTATGACACCTGCATGGGGGCGA AAGTTGAGGCGATAGCGCATGAAGCCCAGCAATCATCCCACTTTC TGGAGACTGTGCGCGGCGTGGCAGCGGTAAAAGTGTTTGATTTA GCTGAATACCGGCGTAACGCATGGCTTAACCGGGTTATTGATACC GCGAATGCACGCGCTCATCTGTTAAAGATAGATCTTATTAACCAG ACGCTTTCGGCTCTGCTGACGGGTCTCTCATCGGCAGCGATCCTG TTTATCGGCGGCAGCCTGATGGAAGCGGGCATAATGACCGCGGG TATTCTGTTGGCTTTTCTGCTCTATGCAGATATGTTCCTTACCCGT TCAGTGAAGGTGATAAATTCGCTGTTTGATTTTCGTCTGATCTCGA TCCACACGCAGCGCCTGACAGATATTGCTGCAACCGAAACAGAAA GTGCATGGAATCCGCTAAATCCTGTACGGCTTGAGAACGTATCCG GCCAGCTAACCCTGAGTGCGCTTTCATTTCGCTACAGTGAGGCGG AACCCTTTATTTTCGAAGGGATAGATATGGAGATCAAACCGGGCG AGAGCGTAGCGATTATCGGCCCATCAGGCTGTGGTAAATCGACG CTTCTCAATGTTATGGCGGGTCTGACTCTTCCGCATTCAGGAGAG ATATTTATTGATGGCGTTAGTGTCCGCCAGACTGGTATTGACGAA TACCGTCGGCACACGGCGTTTGTCATGCAGGATGATAAATTATTT GCAGCCTCACTCATGGATAACATCACTTCTTTTACCCCACAGCCTG ATATTGACTGGATGCATGAATGCGCCACGGCAGCGGCAATCCAT GATGAGATTATGGCGATGCCGATGCAATACGAAACGATGGTGGG TGACATGGGAAGTATTCTTTCTAGCGGACAAAAACAGCGCGTGTC GCTCGCCAGGGCGCTGTACAAGCGTCCCCGCATTCTGTTTCTTGA TGAGGCCACCAGTGACCTGGACGTTATTAACGAGCGGAAGATCA ATGAAGCGGTAAAACAGATGCCTGTTACACGGGTATTCGTGGCTC ACCGGCCAGAGATGATTGCTGTCGCCGATCGGGTTTATAACCTGA GAGATAAAACTTTTGTGCCATCAGGCTATGAGGTTACAGATTAA (SEQ ID 200)

pacAB (Protein ID: WP_072023203.1, WP_036768348.1)	pET-28a(+)	NdeI_XhoI	TCTAACTTGAAAAAAGAAATCGCTGAAACTAAAACTGAAATTAAAG GTACTAAAGTTAAAAATAATCAACCTCAACCTCTAACAGAAGATCT GCTCGACCAAATCTCTGGTGGTTGGGTGAATGCTTACGCAAGATG GACAAACCGCTTTTAAattcagtagattaaagtcaggggcttaattgccccca tttgattctttcgagctgagcaatgttcgtagttggaacttaacctgccattttcgtattac tggcatagggtctaacaaagtaaaaaATGGAGCTTCGAGTGATGGTTAAT TCATTAGTTAAGAAAAAAATTCAACATCTTGAAGTAATATTAAAGA TAAGCGAGCGATGTAATATCAATTGTGACTATTGTTACGTATTCAA TAGAGGAAATTCAGCGGCTAATGATAGCCCCGCCAGGATCTCTCA TGCGAATATTGATTACCTGGTGGATTTCTTTCAGCGGGGAAGTCA AGAATATGATATTGACACTCTGCAAATTGATTTTCATGGAGGAGA ACCTCTCATGATGAAAAAGCCGCAGTTTGCCAGTATGTGTGAGCG ACTAGCCTCAGGTAATTACCATGGTTCGAAAATCAGATTTGCATTA CAGACTAATGGCATCCTTATTGATGATGAATGGATATCTTTATTCG AAAAATATTCTGTCAGTGTGAGTGTCTCCATTGATGGACCGAAGC ATATTAATGATCGTCATCGCTTAGACAGAAAAGGGCGTAGTACTT ACGAAGGTACTATACGGGGTCTCCGTAAACTTCAAGAAGCTTATC AAGCAGGTCGGCTGCCGTCAGATCCGGGTATTTTGTGTGTCGCG AATGCTAAAGCAAGCGGGGCTGAAATATATCGACACTTTGTTGAT AACCTGGGCGTTTATGGCTTTGATTTTCTGGTACCTGACGACTGT TACACTGATGCCCAGGTTGATCCAGATGGCGTTGGACGTTTCCTA AATGAGGCGTTAGATGAATGGGTGAATGACAATAACCCCAAGATT TTTGTGCGTCTTTTTAATACCCATATTGCCAGTCTTCTTGGCGCGG AAAATGCGGGGTTTTTGGGGCATAACCCAAGCGTAGCTGGAATAT ATGCATTTACCATTGGTTCAGATGGTTTTGTCCGTGTCGATGATAC CTTGAGATCGACATCTGACCGTATTTTCGACATCATTGGTCACATT TCTGAAATCAGCCTATCTGAAGTATTAAATAGCCCACAGTTTCAGG AATATGCGTCTATAGGGGAATCGTTACCAACAGAATGTGAAGACT GTATTTGGGCAAAAGTTTGTGCCGGTGGGCGCATAGTTAATCGCT TCTCGCATGAAGAGAGATTTAAACGCAAGTCAGTATATTGTTATTC AATGAGAAGCCTTCTTAGCCGCGTTTCAGCTCATCTTCTCAATATG GGGATTGAGGAAGATCGCATTATGAAAGCGATTGGCCGGTAA (SEQ ID 201)

pacDEC (Protein ID: WP_051690838.1, WP_036768349.1, WP_110882651.1)	pCDFDuet-1	NdeI_XhoI	CCAGTAGGCGCCTCAGTTTGGACAATAATAGCGCTTGTTATTATT GTCAGCCTTGTTGTGTTCATGATAATAGGCACTTACACACAGAAG GTTCGGCTAATGGGGGAAATTATCTACGAGCCTGCGGTTGCGAG AATAGAAGCAACGGGTAACGGAACCATTGTCCGTAGTTTTGCTGT TGAAGGGAAAGAAGTTCGCGCTGGAGATGTTATTTTTATCGTTAA CATGGAAACTCAAACCGAATATGGGCGTACAAGTCATGAAATTAC TTCTGCCCTCAAGTCACAAAAAACCGCTATTGAACGAGAGATCAT GCTGAAATCAGAGGCGTCTGATCAAGAAAGTGATTTTCTTACCCA GCGTCTTAAGAATAAGGAAGCGGAAATTCAAGAATTAGACAACCT GATCACAAAATCAACCGAACAAGTCGCGTGGCTATTTGACAAAGC TCAGCTTTTCAATAAATTAGTTGGGAAAGGAATCGCACTTGAAATA GATCATATAGAACGCCGCTCTGATTATTATACTGCTTCTGTTCAAC TGGCGGCTTACAAACGAGAAAAGGTTAAGTTACAGGGTGAATCTC TCGATATCAGGGCGAGGTTGGCGACAATCCACATTGGACTTGAAA CTTCACGTGAAACATTACGTCGAGATATTGCACGGCTAGATCAAG ACTTAGTCTCTACGGCAGAACGAAGGGAACTCTATATAACGTCTC CAATTGACGGTAAGTTAACGGGAATTACTGGATTAGTTGGCAAAA GAATTCGCTCGTCCCAGGAATTAGCGAGTGTTGTACCTACTTCGG GCCGCCCCAAAGTAGAAATCTTTTCCACTTCTGAAGTTATTGGAG AATTACGCGAGGGACAATCTGTAAAATTACGGTTTGATGCTTATC CATACCAGTGGTTTGGGCAGCATGATGGTATTGTTACTGCAATTT CCACGACTTCAGTTGAAGGGAGTTTAGGAATAAAGGATGAAAATA ATCAGCAACAGAAACGGTATTTTCAGGTTCATATCCGTCCTAAAA GCGACGGTGTACTCTTAGCGGGAAATATGCATCCTTTACGGCCCG GAATGGGGGTCGAAACAGACATTTTTATAAGAAAAAGGCCAATCT ACGAATGGATTTTGTTACCTCTAAAAAGAATTCATGTCGCGACTCA AGGTAAACCTGGAGATGATGTATGAATGTCACAATGAAAGGCTAC TTTGAAGCATTCAGGCACCATCTTCCTGTAGTGATGCAAACAGAG GCTACGGAATGTGGACTCGCTTGTGTCGCTATGATTGCAGGTTAT TATGGACTTAATATGGATCTGCAAGCGCTTCGCAAATATTATCAG GTGTCTTTAAAAGGTATGAACCTGCGCGATATTATCGTATTAGCT GATCGCCTCTCATTAGCGTCTCGTCCAATTCGAGCTGATCTTGATT CTTTAAGTCAGGTAAAAACGCCTTGTATTTTGCATTGGTCTTTTAA TCACTTTGTTGTATTAAAGAAATTTTCACGCCGTGGGGTCGTTATT CACGATCCGGCAAAAGGCGAGAGAAGAATTTCTATCGATGAGTTA TCTAAAAAATTTACGGGTATTGCACTTGAGCTTTGGCCAAATAAAG ACTTTCAGAAACGTACTGAAAAGAAAACAATTCGACTGCTGGATA TGTTTAAAAACGTTTCTGGATTATCTCGGGCTTTAGTTCAAGTATT GGCTTTATCATTTTGTATTGAGCTTCTTGCTATGGCCGTGCCGATG GCAGCTCAATTCACGATAGATATGGCTTTGAGGTCTAGCGATATT GATCTTGTCTCTGTGATTGTGTGCGGAATTATTGGCTTATTAATAT TCAGAGCCTTGCTTAGTATTGTGCGTTCATGGTCTGTTATGTCGAT TAAGTATACTTTGGGTATTCAATGGAGCTCTGGGCTTTTTAGTCAT ATGATCCGATTACCTACTTCATACTTTGAAAAGCGTCATATTGGTG ACGTCACTTCGCGATTTAACTCTTTATCGGCAGTACAAGATGCCTT CACCGCGGATATGATAGCTTCACTCTTAGACATTGTTGTGGTGAT TGGACTCTTCTTTTTAATGTGGGTTTACAATGGTTATCTTGCTGTC GTGGTCATTTCGATATCCATTGTATACGCATCGCTAAAATTCTTTC TTTTTCGAGCCTATCGTTCGGCTAATCTCGAGGCGATAGCCCATG AATCTCAGCAACAGTCACACTTCCTTGAAACAGTACGCGGCATCA CTTGCGTTAAAATTTTTGACTTAGCCGATCGCAGACGATCCGATT GGCTCAATCTTGTTATTGATGAAGCCAATGCAAAAATATACCTCTT TAAAATTGACCTGGTGACACAGACTGCGGCACAGCTTTTAATTGG TCTTACTTCTGCATCCATATTATGGTTAGGCGCTAAATTGATTGAT

			GGCGGCGCGTTAACCACAGGTATGCTTTTTGCCTTCTTGATTTAC TCTGATATGTACGTAAATCGAACCATACGAGTGGTTGACTCGATT ATTAAACTTCGCTTGATCGATATGCATAGCGAACGACTGTCAGAA GTGGCTTTAGCCGAACCTGAACATAATGAAGGGGATGCTGTTCTA TCATGTCCTGAAACAATTTCAGGCAGTATTGAAATTAAAAGCCTGA GTTATCGTTATGGCGATGGCGAACCCGCTATATTTGAGAATGTTT TTCTGTCTATTAAGGCTGGTGAAAGTATCGCTATAGTTGGGCCGT CAGGTTGTGGTAAATCGACACTGCTTAAGACAATCGGTGGATTAG TCTCGCCAGAAAGTGGCTTTATTTATTTGGACGGAGTTGATGTGC GGAGATTAGGACTTGGGGCCTACCGTAGCCATATCGCTTGTGTCT TACAAGAGGACAGATTATTTGCGGGATCGCTATTGGATAATATTA GTTCATTCGACGTTAAGCCTGACCATGAATGGGTATATGAGTGTG CTCGTCTTGCTTCAATTCACGCTGAAATAGAAGAGATGCCAATGA AATATGAAACAATGGTTGGAGACATGGGCAGTGCTCTGTCAGGT GGACAACGGCAGCGTATTTCTCTTGCCAGGGCATTGTACAAACGT CCAAAGATATTATTTCTTGATGAAGCAACGAGTGATCTGGATATC GATAACGAAGCAAAAATTAATGACTCAATACGAGAACTAAAGATT ACCAGGGTATTTGTAGCCCATCGTCCGACAATGATCGCAATGGCG GATAGGGTTTTTGATCTAAGTATGAACGCAGAAGTGGAGAACCCC CATGCATTTTTCTCTAAGTAAACATATCAAGGTGACCGCATTTGTT GCTTTTTCTTCCATGATGTCATTATTTGTTGCAAATTCTATGGCCG CTGAAAAAGTCATGCATATCAATTTTCAATTTGATGAATTTGCTCT ACCGATAGCAAATCTTGAAATTGATGGAAAAACTCAAAATCTTATG ATCGATACGGGTTCAACTATAGGTCTCCATTTATCTAAAAACCTGA TGTCGAAAATTTCCGGCTTAGTTATCGAACCTGAAAAAGCGCGTT CTACTGACCTTACGGGTAAGACTTTTTTAAATGACAAATTTAATAT TCCACGGCTTTCGATAAATGGCATGATGTTTAAAGATGTTAAAGG GGTTTCATTAACACCATGGGGAATGAAATTAATTGGAGACAATGA TCTTCCTTCCTCAATGGTAATTGGCCTTGATTTATTCAAGGGAAAG GTGGTTCTTATTGATTATAAAAGCCGGAAATTATCAGTTTCTGATC GTTTGCAAGCGTTGGGAGTCAATGTGGATAATGGTTGGATAAAAT TGCCGCTGAGACTGACTAAAGAAGGCATTGCTGTCAAAGTTTCAC AAAACTTTAAAAGCTACAACATGGTATTGGATACTGGCGCATCGG TTTCGATTTTTTGGAAAGAAAGATTGAAATCTCCTCCGGTTAACAT TTCTTGCCAGGCTGTGGTTAAAGAGATGGACAATGAAGGGTGTGT TGCATCGACGTTTCAGCTTGACGAAATGGGCGTTAAGGGAGTTAA GCTGAATTCGGTATTGGTTGATGGGGGATTTAATCAGTTAAATAC TGATGGATTAATCGGGAATAATTTCTTTAATAAATACGCAGTATTA ATCGACTTCCCTGGTAAGAGATTATTCATTAAAGAGAACTCGTAG (SEQ ID 202)

xyeB$_{24}$-*xncCDE* (Protein ID: WP_103774053.1, WP_013185693.1, WP_013185694.1, WP_013185695.1)	pCDFDuet-1	NdeI_XhoI	GCTAACAAAGAAAAAATCAAACACCTGGAAATCATCCTGAAAGTT TCTGAACGTTGCAACATCAACTGCACCTACTGCTACGTTTTCAACC TGGGTAACGACCTGGCTATCAACTCTAAACCGATCATCTCTCACG GTACCATCAAAAACCTGCGTGGTTTCTTCGAACGTGCTTGCCAGG AATACGAAATCGAAACCGTTCAGGTTGACTTCCACGGTGGTGAAC CGCTGATGATCGGTAAAGACCGTTTCGACAACGCTTGCAAAGAAC TGGTTTCTGGTGACTACAACGGTACCCGTCTGAACCTGGCTTGCC AGACCAACGCTATCCTGATCGACAACGAATGGATCGACATCTTCT CTAAACACAACATCTCTGTTGGTATCTCTATCGACGGTCCGAAAC ACATCAACGACCGTCACCGTCTGGACCGTAAAGGTCGTTCTACCT ACGAAGGTACCGTTAAAGGTCTGGAAATGCTGCAGGCTGCTTGG CGTGCTGGTCGTCTGATCGACGAACCGGGTATCCTGTGCGTTGCT AACCCGTCTGTTAAAGGTGCTGAAATCTACCGTCACTTCGTTGAC GTTCTGAAATGCAAAAAATTCGACTTCCTGATCCCGGACGAATCT CACGACACCTGCACCGACCCGGAAGGTCTGTCTGACTTCTACTGC TCTGCTCTGGACGAATTCTTCCTGGACGCTGACAAAGAAGTTTAC GTTCGTTACTTCCACACCCACATCCAGTCTATGCTGTCTCTGGAAT TCTCTCCGGTTATGGGTGTTTCTAAAGCTGGTTCTGACACCCTGG CTTTCACCGTTTCTTCTGACGGTGAACTGTACGTTGACGACACCC TGCGTTCTACCAACGACTCTATCTTCACCCCGATCGGTCACATCCA GTCTCTGACCCTGTCTGAAGCTCTGACCTCTTGGCAGATGCAGAA ATACCTGTCTGTTGACAACCAGCTGCCGGAAGTTTGCATCGACTG CATCTGGAAAAAACTGTGCGGTGGTGGTCGTCACATCCAGCGTTA CTCTTCTGCTGACGACTTCAACCGTGAAACCGTTTTCTGCCCGTCT ATCCGTAAAATCATGTCTCGTGCTGCTTCTCACCTGATCGAATCTG GTGTTACCGAAGACATCATCATGAAAAACCTGGAAGTTAACTCTT AATGGAGCCGGACAATGGAAAAAATCAATTTCTGGTTATCAAAGT TTTCATGTGCCGCCCTCGCTATTTGTTGTACATCTTGCCTTGCTGA CTCGGGAAATTCGGTAACACTTAAGCTGAATTATGACAAATATTTC ACGCCTCATGCAACTTTCATCATTAATGGCCACCCGGTAAATATG ATGATTGATACAGGTTCTTCGAAGGGCTTTTATCTTCAAGAGCCTC AACTAAAAAAAATACAAGGCCTCAAAAAAGAAAGCACTTATTACA GTACTAATATCACCGGGAAAAGACAGGAGAACACAGAGTATCTCG CCGCTTCTCTCGACATGAATGGCCTTAAATTAAAAAACGTAACCGT GATCCCATTTAAACAATGGGGAGCGCTGATTTCTAACACAGGTAA ATTGCCGGATGGCCCTGTTGTCGGTCTCGATGCGTTTAAAGATAA ACAAATTATGCTGGATTTTGTGTCTCATTCATTCACGATGAGCGAC AGTTTTATCCATAACATGCCGGTTCCGAAAGGCTTTAACGCATTCA CTTTCCATATGTCTCCTGATGGCATGGTTTTTGATGTTGATCAGTC TGGACACACATACCATTTGATTCTGGACACCGGTGCCACTGCGTC TGTGATTTGGCGTGAAAGACTTAAACAGTATGAACCCAAAAGCTG CCTGCTGGTCGATCCGAAGATGGATAACGAAGGATGCCAGGCCA CTCTGCTCACAATTAAATCAAAAACTGGAAATCCCCAGCATTTTGG TGCGGTTGTTGTTGTCGGAAATTTTAAACACATGGGCAACGTTGA TGGCCTTTTAGGGAATAACTTCCTCAGAAATCGAAAGGTACTTATA GACTTTAAAAACAAGAAGGTTTTTATTTCCGATGAGCACCGAAAC AGAAAAGAATGACAACTCAATCTTTCGTGCCGAGGCTTTGCAACA CAAACGAGAAGGTTGGCTCGGCGCTTCTCGTTTGCATATACCGTC AGCGCTCTCTATTTGTTGCCTGACAATCCTTGTTATTTTCTTTTTCA TCATATTGATAATTGCATTTGGTTCGTACAGTGAACGGATAAATGT CATCGGAACCGTGGTTTATAAGCCGCCTGCGGTATCACTGATTGC ACAAAGCAGTGGAATCATTACGCATTCACTGGCATTAGAGCAAAC AAGAGTTAAGCGCAACGAGAGCATTTTTTCTATCAGTGGTGACAC TCAGACAAATCTGGGTGCCACCAATGTTGAAACGGTAGAACTTTT

			AAATAAGCAACGTAACGCGCTGTCTAAAAAGCTTGATATTGCGGC CAATGAATCAAAAGCAAACAAGATTTATCTCAGCGAAAAAATTAAA AATAAACAACAGGAAATAGAAAGTCTGCAAAACCTGATAGAAACT TCAGAAAAACAGCAAGCGTGGTTCGAGAAAAAATCAAACCTGTAT GCGAATTTTAAGAAGAAAGGCATTGCGCTTGATGCTGAATGGATA AACAGAAAGAAAGATTATTACGCATCCACATTAAGCATTTCTTCTG CAAAGGTCAAAGTGATAGCCCTGCTGGGAGAGTTGCAGGATCTG AAAAATGACGTTTCGGTTATCGACAGGAAACTCGACAAAGAAACA GCATCTCTCACTGTCGAAATAGCCGATATAGCACAAAAAATACTG ATTACAGAAAAACAAAAAGAGTATTTAATCGTCGCGCCGTTTGAT GGAATGATAACCAGTGTTACAGCCCATATCGGTGAAAGAGTGACT GCCGGCCAGCAAATAGCCGTGCTGATACCACAAGGTGCGACAGA AAAGGTTGAGTTGTTTTCACCGTCTGATTCTCTCGGTGAAGTGAC CAGCGGACAGCAAGTCAGAATGAGAGTCTCGGCATACCCTTACC AGTGGTATGGAAAGATTGCAGGCATCATAGAAACGATATCGGCA GCACCGGTCAATGTCACCTCACAGATGCAGATGAAAGGTGAAGA GGTAAAAAAGGGGCTTTTTCGGATTGTCGTACAACCAAAATTGAC CGGACAACAAACAAACATTTCCCTTCTACCCGGCATGGAAGTGGA AACAGAGATCTATGTGAAAACCCGAAAATTGTACGAATGGTTATT TATCCCCATTAAAGGGGCATATGAACGGGCGACAGACAGTACGG AATAAATATGCAGTATAAGATGAGTGATTTTTTCGAGTTTTTCGTC AAAAAACTCCCGGTGATAATACAAACAGAGACCACAGAATGCGG GTTGGCATGTCTGGCCATGATTGCTGCCTGGTATGGCCGTGAGA CTGATATCTACAGCATGAGAAAGGTTTTTGACGTGTCAAACAATG GCATGACATTAAGGCAGATCATCACGGCGGCCGGGCGAATAAAC ATGAATACCAGAGCTGTGCGGCTGGAACTCAACGAACTCAGCAG TGTCAGGCTTCCGTGCATCTTGCACTGGTCCTTTAATCATTTTGTC GTGTTAAAAAAATTCACAAAAAAAGGGGCAGTCATCCATGATCCC GCCTTGGGAAAAAGAACTGTCACTCTGAAAGAACTCTCAAATAAG TTTACGGGCATCGCTCTGGAAGTCTGGCCCCAGACGGAGTTTAAA AAGGAAAAGGTCAGTGAAAGCATAACCATCACGGATATGTTTCGC GGTGTTGCCGGCCTTAAGAATACGCTGTTTAAAATCATTCTGTTGT CGCTCTTTATTGAAGTACTGGCACTTTCCATCCCTCTCAGCTCTCA ATTCATTATTGATGTTGTTCTACGGTCCAGTGACCTCAGTATGCTG AATTTCATTGTCATTGGAATCGTTCTTCTGCTCTCCCTGCGCGCTG CTTTCAGTATTGTGCGCGCCTGGGCTCTTATGGCAATGCGTTACT CACTTGGCATACAGTGGAGTTCCGGTTTTTTTAACCGGTTACTCA GATTGCCGGTCACTTTTTTTGAAAAACGTCACGTAGGTGATATCG CCTCCAGATTGACATCGTTGAGCGAAGTTCAAGAAGCCTTTACAG CAGAAATGCTGACTTCGTTACTTGATGTACTTATTCTCATAACGCT GGCTGTGCTCATGTTCTGTTACAGCCCTCTTCTGACCCTTCTCCCG CTACTCATGACTACCGTTTATCTTGGGGTCAAATTTGCTTTTTATG ACAGATACATGGGAGCAAAAGTAGAAGCAATTACGCATGAAGCG CAGCAATCATCCTACTTTCTCGAAACAATACGAGGCGTAGCGTGC GTGAAAGTATTTGGCCTGACAGAATTCCGACGTATCACATGGCTT AACCGGGTGATTGATACTGCCAATGCCCGGGCCCATTTATTTAAG ATAGACCTCATCAGCCAAACGCTTTCAGGTTTCCTGACGGGGCTA TCATCGGCGGCCATTTTGTTTATGGGGAGTCATCTCACAGAACGC GGCCTGATCACTGCCGGCATTCTGTTTGCTTTTCTGCTCTATACCG ATATGTTTCTGACACGTTCAGTGAAGGTAATAAATTCACTGTTTGC TTTTCGCCTTATTTCGATACACACGCACCGATTGACCGATATTGCA ACAGCCCAGACAGAAAATGCATGGAACCCGGAAGATCCCGTCAC ACTCGATAATGTAAAAGGCCGGATAACACTGAACAATCTCACATA TCGGTACGGAGAAACTGAACCCTGTATTTTCGACTGTATCGACAT

			GGAAATTAATGCTGGTGAGAGTGTGGCGATCGTAGGTCCGTCAG GTTGCGGTAAATCGACACTTCTCCGGGTCATGGCCGGCCTGGTTC TCCCTCAGTCAGGCGATGTGTCAATTGATGATGTCAGTGTGAAAA AAATGGGTATTGACGAATATCGCAGACACACGGCGTTTGTCATGC AAGATGATAAGCTTTTTGCTGCCTCATTGATGGATAACATATCCGC TTTTGATCCACAGCCAAATATTGATTGGATACATGAATGCGCTAAG GCGGCGGCAATACACGATGAAATTATGACTATGCCGATGCAGTAC GAAACCATGGTGGGTGACATGGGGAGCATTCTTTCAGGCGGACA AAAACAGCGTGTATCCCTTGCACGGGCACTTTACAAGTGTCCGCG TATCCTCTTTCTTGATGAGGCCACCAGCCATCTCGACGTTTTTAAT GAACGCAAGATAAATGAGGCTGTAAAGCAGATGCCGATTACGCG TGTATTTGTGGCTCATCGGCCAGAAATGATCGCTGTCGCAGACCG AGTTTATAACCTGAGGGA (SEQ ID 203)
xyeA$_{24\text{-}1}$ engineered	pET-28a(+)	NdeI_XhoI	TCTAAACTGGCTAAAGAAATCTCTATGAACAAAGCTGCTGTTATCA TCGACGGTGACAAAAAAGACGTTCGTCGTGCTCTGACCCAGTCTA TGCTGGACTCTGTTTCTGGTGGTTGGGTTAACgcaTTCGCTCGTTG GTCTaaaCGTTGGTAAAATTCGAGCTCGGCGCGCCTGCAGGTCGA CAAGCTTGCGGCCGCATAATGCTTAAGTCGAACAGAACCCAAGAC CAGGGGGGCTCGCCACGTTGGCTAATCCTGGTACATCTTGTAATC AATATTCAGTAGAAAATTTGTGTTAGA (SEQ ID 204)
xyeA$_{24\text{-}2}$ engineered	pET-28a(+)	NdeI_XhoI	TCTAAACTGGCTAAAGAAATCTCTATGAACAAAGCTGCTGTTATCA TCGACGGTGACAAAAAAGACGTTCGTCGTGCTCTGACCCAGTCTA TGCTGGACTCTGTTTCTGGTGGTTGGGTTAACgcaTTCGCTCGTTG GTCTaaaCGTttcTAAAATTCGAGCTCGGCGCGCCTGCAGGTCGAC AAGCTTGCGGCCGCATAATGCTTAAGTCGAACAGAACCCAAGACC AGGGGGGCTCGCCACGTTGGCTAATCCTGGTACATCTTGTAATCA ATATTCAGTAGAAAATTTGTGTTAGAA (SEQ ID 205)

His6-ykcA + ykcB (Protein ID: WP_072082693.1, WP_050115763.1)	pRSFDuet-1	NcoI_XhoI	GGTCATCACATCATCATCATCATCACAGCTCTGGATTAGTGCCGC GCGGTAGTCATATGTCTCGCTTACAAAAAGAAATCAATGAAACTA AGACAGTCATTAACATTTGTAATACTAAAAAGAGTCAACCTCAGCA TCTTGCAGACAGTATTCTCGACAAGATAGCAGGCGGTTGGGTGAA TGCTTTTGTAAACTGGCCAAAAAGTTTTTAAgaattcgagctcggcgcgc ctgcaggtcgacaagcttgcggccgcataatgcttaagtcgaacagaaagtaatcgt attgtacacggccgcataatcgaaattaatacgactcactataggggaattgtgagcg gataacaattccccatcttagtatattagttaagtataagaaggagatatacatATGG TCAATCAATTAAACATTCAAAGCATCCAACACCTTGAAATAATATT AAAAATAAGCGAACGCTGTAATATTAATTGTGATTATTGCTATGTA TTCAATAAAGGTAATCCGGCGGCTAATAACAGCCCCGCCAGATTG TCAGATAGAAACATTAATGACTTAGCTGAATTTCTTCACACAGCAT GTCGGGAATATAAAATCGGTACCCTACAAATTGATTTCCACGGGG GGGAACCGTTATTGATGAAAAAAGAAAACTTCGCCAAAATGTGTG AGCGATTACTGACAGGAAGATACTCGAAGACTAATATCAGATTCG CATTGCAAACTAACGGCACACTTATTGATGAAGAATGGATATCAC TATTTGAAAAATATTCTGTGAACGCAAGTATTTCTATTGATGGCCC GAAACATATTAATGACAGGCATCGTTTAGATACCAAAGGGCGTAG CACTTACGAGGCGACAGTGCGTGGTTTGCGTATACTCCAACATGC TCATAAGCAAGGCCGTATTCCATCGGCACCGGGGGTTTTATGTGT CGCGAATGCTCAAGCAAATGGTGCTGAGATATATCGTCATTTTGT GGACGAATTAAAGGTTTATGGTTTTGATTTTCTGGTGCCAGACGA TTGTTATCATGACACTAATATTGACCCTGTTGGTATTAGCCGCTTC CTAAATGAAGCTTTGGATGAATGGTTCAAGGACAGCAACCCTAAT ATTTTTGTCCGCCTTTTTCAAACACACTTAGCTCATTTGCTCGGTA CAAAGCATCAAGGAATTTTAGGGCATTCACCCAGCGCCACTGGG GCATACGCATTCACCGTGGGTTCAGATGGTTTTATTCGTGTGGAT GATACCTTACGCGCCACATCAGACAGAATTTTCAATCCCATTGGT CATGTTTCTGAAATCAGCCTAACTGATGCACTTAATAGCCCTCAGT TCCAGGAGTACGCGTCAGTCGGCCAAGCTCTGCCCCATGAATGC AACGGTTGCATTTGGGAAAACGTCTGTGCTGGAGGTCGTATTATG AATCGTTTTTCACCTGAAACCCGCTTCGACCGCAAGTCTGTTTATT GCTATTCCATGAGAAGTTTCCTCAGCCGCGCCGCTGCACACCTAC TCAATATGGGCATCAAGGAAGAGCGCATTATGACAGCAATTGGG CGATAA (SEQ ID 206)
$xncA_L$-$ykcA_C$	pET-28a(+)	NdeI_XhoI	AGCAAATTACAGCGTGAAATTGCAGCAAACAAAGCTCAACTGAGC CATGAAGACAAGAAGAAAACGCAGCACAAAGAGCTTGTTGACAG CCTGC 42 TGGATACTGTCTCTGGTGGTTGGGTTAACGCTTTCGTTAACTGGC CGAAATCTTTCTAA (SEQ ID 207)
$xncA_L$-$xecA_C$	pET-28a(+)	NdeI_XhoI	AGCAAATTACAGCGTGAAATTGCAGCAAACAAAGCTCAACTGAGC CATGAAGACAAGAAGAAAACGCAGCACAAAGAGCTTGTTGACAG CCTGCTGGATACTGTCTCTGGTGGTTGGGTTAACGCTTTCGCTAA CTGGTCTAAATCTTTCTAA (SEQ ID 208)
$xncA_L$-$socA_C$	pET-28a(+)	NdeI_XhoI	AGCAAATTACAGCGTGAAATTGCAGCAAACAAAGCTCAACTGAGC CATGAAGACAAGAAGAAAACGCAGCACAAAGAGCTTGTTGACAG CCTGCTGGATACTGTCTCTGGTGGTTGGGTTAACGCTTTCGCTCG TTGGGACAAAAAATTCTAA (SEQ ID 209)

xncA$_L$-*ajcA*$_C$	pET-28a(+)	NdeI_XhoI	AGCAAATTACAGCGTGAAATTGCAGCAAACAAAGCTCAACTGAGC CATGAAGACAAGAAGAAAACGCAGCACAAAGAGCTTGTTGACAG CCTGCTGGATACTGTCTCTGGTGGTTGGGTTAACGCTTTCGCTAA CTGGACCAAACGTTTCTAA (SEQ ID 210)
xncA$_L$-*phcA*$_C$	pET-28a(+)	NdeI_XhoI	AGCAAATTACAGCGTGAAATTGCAGCAAACAAAGCTCAACTGAGC CATGAAGACAAGAAGAAAACGCAGCACAAAGAGCTTGTTGACAG CCTGCTGGATACTGTCTCTGGTGGTTGGGTTAACGTTTTCGCTCG TTGGGACAAACAGATCTAA (SEQ ID 211)
xncA$_L$-*vscA*$_C$	pET-28a(+)	NdeI_XhoI	AGCAAATTACAGCGTGAAATTGCAGCAAACAAAGCTCAACTGAGC CATGAAGACAAGAAGAAAACGCAGCACAAAGAGCTTGTTGACAG CCTGCTGGATACTGTCTCTGGTGGTTGGGTAAACGCCTTCGCACG CTTCACGAAGCGCTTCTGA (SEQ ID 212)

[a]Small letters indicate untranslated region.

In some embodiments, the nucleic acid molecules are introduced into the host cell via a pET28a(+) vector and/or pCDFduet-1 vector. In some embodiments, the nucleic acid molecules are introduced into the host cell via a pET28a(+) vector, pCDFduet-1 vector, pACYCDuet-1 vector, pETDuet-1 vector, pCOLADuet-1 vector, pRSFDuet-1 vector, pBAD vector, or a combination thereof.

In some embodiments, the host cell is E. coli NiCo21(DE3) cell. In some embodiments, the host cell is E. coli NiCo21(DE3), BL21(DE3), BL21-AI, BL21 Star™ (DE3) pLysS, Rosetta™(DE3), or a combination thereof.

Through the method described above, the polypeptides obtained may be distinct from each other. These polypeptides are then tested for the desired properties. In this way, resources can be preserved as polypeptides having the same chemical structure is not tested.

The present invention also provides a method of producing a polypeptide, the method comprising:

a) expressing a precursor polypeptide and a rSAM/SPASM maturase;

wherein the precursor polypeptide comprises a first three residue motif (from a N-terminus) and a second three residue motif, the first and second three residue motif optionally separated by 1 to 3 amino acid residue, and at least two C-terminus residues;

wherein the three residue motif is each represented by X_1-X_2-X_3;

wherein each X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine, an unnatural aromatic amino acid residue or a derivative thereof;

wherein each X_2 and X_3 are independently any amino acid residue;
wherein at least one of the two C-terminus residues is an aromatic residue;
wherein the rSAM/SPASM maturase is capable of modifying the precursor polypeptide to form a polypeptide with a cyclophane moiety connecting the X_1 and X_3 residues in each motif.

In some embodiments, the method further comprises contacting the polypeptide of step a) with a protease.

The present invention also provides a method of producing a polypeptide, the method comprising:
a) expressing a precursor polypeptide and a rSAM/SPASM maturase in order to form a modified precursor polypeptide; and
b) cleaving the modified precursor polypeptide from the rSAM/SPASM maturase using a protease to form a cleaved modified polypeptide;
wherein the precursor polypeptide comprises a first three residue motif (from a N-terminus) and a second three residue motif, the first and second three residue motif optionally separated by 1 to 3 amino acid residue, and at least two C-terminus residues;
wherein the three residue motif is each represented by X_1-X_2-X_3;
wherein each X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine, an unnatural aromatic amino acid residue or a derivative thereof;
wherein each X_2 and X_3 are independently any amino acid residue;
wherein at least one of the two C-terminus residues is an aromatic residue;
wherein the rSAM/SPASM maturase is capable of modifying the precursor polypeptide to form a modified precursor polypeptide with a cyclophane moiety connecting the X_1 and X_3 residues in each motif.

This allows the method to be more versatile as a commercial protease can be used to cleave the modified precursor polypeptide *in vitro*.

In some embodiments, the protease is derived from *Xenorhabdus Spp.* In some embodiments, only the protease is derived from *Xenorhabdus Spp.*

In some embodiments, at least one motif comprises X_1 and X_3 connected via phenylene to form a cyclophane moiety. In some embodiments, at least one motif comprises X_1

and X_3 connected via indolylene to form a cyclophane moiety. In some embodiments, the two motifs separately comprises phenylene and indolylene. In some embodiments, the X_1 and X_3 in the second motif are connected via phenylene to form a cyclophane moiety.

The present invention also provides a method of synthesising a polypeptide as disclosed herein, the method comprising:

(a) coupling a pre-sequence peptide to a support, wherein said pre-sequence peptide comprises amino acid residues having side chain functionalities which are, if necessary, protected during the synthesis;

(b) coupling one or more N-protected amino acids to the N-terminus of the pre-sequence peptide to form a precursor polypeptide, wherein each coupling is performed in stepwise fashion and under conditions in which each of the amino acids of the target peptide is coupled and subsequently N-deprotected;

c) cleaving said precursor polypeptide from the support; and

d) synthetically or enzymatically connecting the X_1 and X_3 in each motif to form a cyclophane moiety.

The step of d) connecting the X_1 and X_3 in each motif to form a cyclophane moiety can occur before the cleaving step c). In this regard, the modification of the precursor polypeptide can occur on the support.

The step of d) may be performed synthetically. For example, the precursor peptide may comprise an alkyne moiety and an ortho-iodoaniline moiety. A Larock indole synthesis may be performed to form an indolyene containing cyclophane. Alternatively, the precursor peptide may comprise a halophenyl moiety such that a halo substitution may be performed to form a phenylene containing cyclophane.

The support may be a solid phase material or resin (for example, low cross-linked polystyrene beads) which may form a covalent bond between the carbonyl group and the resin, most often an amido or an ester bond. Alternatively, the synthetic method may be performed without the use of a support.

Accordingly, the method may comprise:

(a) synthesising a precursor polypeptide, the precursor polypeptide comprising a

first three residue motif (from a N-terminus) and a second three residue motif, the first and second three residue motif optionally separated by 1 to 3 amino acid residue, and at least two C-terminus residues, wherein the three residue motif is each represented by X_1-X_2-X_3; and

b) synthetically or enzymatically connecting the X_1 and X_3 in each motif to form a cyclophane moiety.

The present invention also provides a method of modifying a precursor polypeptide, the precursor polypeptide comprising:

a) a first three residue motif (from a N-terminus) and a second three residue motif, the first and second three residue motif optionally separated by 1 to 3 amino acid residue; and

b) at least two C-terminus residues;

wherein the three residue motif is each represented by X_1-X_2-X_3;

wherein each X_1 is an amino acid residue, the amino acid independently selected from tryptophan, phenylalanine, tyrosine, histidine, an unnatural aromatic amino acid or a derivative thereof;

wherein each X_2 and X_3 are independently any amino acid residue; and

wherein at least one of the two C-terminus residues is an aromatic residue;

the method comprising:

enzymatically connecting the X_1 and X_3 residues in each motif to form a cyclophane moiety.

In some embodiments, at least one motif comprises X_1 and X_3 connected via phenylene to form a cyclophane moiety. In some embodiments, at least one motif comprises X_1 and X_3 connected via indolylene to form a cyclophane moiety. In some embodiments, the two motifs separately comprises phenylene and indolylene. In some embodiments, the X_1 and X_3 in the second motif are connected via phenylene to form a cyclophane moiety.

In some embodiments, the enzyme is rSAM/SPASM maturase.

The present invention also provides a composition comprising a polypeptide as disclosed herein.

In one embodiment, there is provided a pharmaceutical composition comprising a polypeptide as defined herein. The pharmaceutical composition may comprise a pharmaceutically acceptable carrier. By "pharmaceutically acceptable carrier" is meant a pharmaceutical vehicle comprised of a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject along with the selected active agent without causing any or a substantial adverse reaction. Carriers may include excipients and other additives such as diluents, detergents, coloring agents, wetting or emulsifying agents, pH buffering agents, preservatives, and the like. Representative pharmaceutically acceptable carriers include any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives {e.g., antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, pp. 1289-1329, incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the active ingredient(s), its use in the pharmaceutical compositions is contemplated.

The present invention also provides a use and/or method of treating a disease. In one embodiment, there is provided a method of treating a disease in a subject, comprising administering an effective amount of a polypeptide or composition as defined herein to the subject in need thereof. Provided herein is also a modified polypeptide or composition as defined herein for use in treating a disease. Also provided herein is the use of the modified polypeptide or composition in the manufacture of a medicament for the treatment in a subject. The disease may, for example, an infectious disease. The disease may be caused by a bacteria, or a bacterial infection.

The term "treating" as used herein may refer to (1) preventing or delaying the appearance of one or more symptoms of the disorder; (2) inhibiting the development of the disorder or one or more symptoms of the disorder; (3) relieving the disorder, i.e., causing regression of the disorder or at least one or more symptoms of the disorder; and/or (4) causing a decrease in the severity of one or more symptoms of the disorder.

The term "subject" as used throughout the specification is to be understood to mean a human or may be a domestic or companion animal. While it is particularly contemplated that the methods of the invention are for treatment of humans, they are also applicable to veterinary treatments, including treatment of companion animals such as dogs and cats, and domestic animals such as horses, cattle and sheep, or zoo animals such as primates, felids, canids, bovids, and ungulates. The "subject" may include a person, a patient or individual, and may be of any age or gender. The term "administering" refers to contacting, applying, injecting, transfusing or providing a composition of the present invention to a subject.

In some embodiments, the bacterial infection is caused by a Gram-negative bacteria. In other embodiments, the Gram-negative bacteria is selected from *Escherichiacoli, Pseudomonas aeruginosa, Candidatus Liberibacter, Agrobacterium tumefaciens, Acinetobactor baumannii, Moraxella catarrhalis, Citrobacter di versus, Enterobacter aerogenes, Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhimurium, Neisseria meningitidis, Serratia marcescens, Shigella sonnei, Shigella boydii, Neisseria gonorrhoeae, Acinetobacter baumannii, Salmonella enteriditis, Fusobacterium nucleatum, Veillonella parvula, Actinobacillus actinomycetemcomitans, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Helicobacter pylori, Francisella tularensis, Yersinia pestis, Vibrio cholera, Morganella morganii, Edwardsiella tarda, Campylobacter jejuni, Haemophilus influenza, Enterobacter cloacae*, or a combination thereof.

Examples of polypeptides and their MIC values are shown in Table 3.

The present disclsoure also concerns a method of killing and/or inhibiting proliferation of bacteria, comprising contacting the bacteria with an effective amount of a polypeptide as disclosed herein.

The present disclsoure also concerns a method of disinfecting a surface, comprising contacting the surface with an effective amount of a polypeptide as disclosed herein. The surface may be a medical device or implant.

In the embodiments that follows, the invention is described in relation to some conditions for consistency to showcase the present invention. However, the skilled

person would understand that the invention is not limited to such.

Example 1: Methodology

A three-step approach for antibiotic discovery was envisioned. In step 1, genomic enzymology is used to identify and assign function to proteins that define a natural product family. In step 2, the natural products are produced using synthetic biology – BGCs are synthesized and expressed in a heterologous host producing the natural products. In step 3, the products are tested for bioactivities against a panel of pathogenic bacteria. Historically, typical bioactivity-guided platforms utilize crude or partially purified extracts, which leads to identification of only the most potent natural products while the minor components or those with less potent activities are overlooked. This workflow is problematic, leads to rediscovery of known compounds, and led pharmaceutical companies to abandon natural product drug discovery programs in the 1980s and 1990s. In the present strategy, chemistry is prioritized so that only molecules which have not been characterized or tested for bioactivity are obtained. This approach yields that targeted compound directly and subsequent MIC values can be obtained for each molecule produced. This workflow solves the problems associated with isolation of known compounds, laborious de-replication, bioactive but minor constituents, and cryptic metabolites.

For example, a chemically-guided workflow is disclosed herein to reveal antibiotic activity for Series A xenorceptides, which are named xenorceptides A1-A10. Fundamentally, this workflow starts from a posttranslational modifying enzyme sequence and ends with a peptide antibiotic (Figure 2). This workflow is demonstrated on triceptides, a relatively new RiPP family with no known bioactivity. In particular, the chemically-guided workflow, named GEnSyBER-A herein, can be used to discover ribosomally synthesized and posttranslationally modified peptide (RiPP) antibiotics. This approach starts from radical SAM enzyme sequence-function space enriched in 3-residue cyclophane forming enzymes. Synthetic biology enabled the production of xenorceptides A1-A10, RiPP natural products associated with the Xye maturase system. Xenorceptides are 12-mer triceptides that contain three separate three-residue cyclophanes. Xenorceptide A2 was found to selectively kill several carbapenamase-resistant Enterobacteriaceae (CRE) with MIC values between 4-8 μg/ml. This workflow can provide unique peptide antibiotics with activities against priority pathogens of interest.

Example 2: Xye maturase system (ABCDE)

For example, the Xye maturase systems encode a precursor (XyeA), rSAM/SPASM maturase (XyeB), protease (XyeC), transporter (XyeD), and protease/transporter (XyeE) (Figure 1a). Bioinformatic analysis revealed 81 XyeA precursors with 56 encoding unique core sequences. The latter represents the total number of different xenorceptides that could be produced. The core peptides contain two or three Ωxx motifs (Ω = Trp, Phe or Tyr) downstream of the conserved GG motif and are classified into 4 types (Figure 1b). Type A is the most prevalent and all Ω residues in the conserved ΩxxxΩxxΩxx sequence are involved in the 3-residue cyclophanes. Xenorceptide A1 (**1**) is a representative of Type 1. Although antibacterial activity was not detected for **1**, it is hypothesized that the diversity in bacterial sources and core sequences within XyeA precursors had the potential to generate peptide antibiotics.

The Xye nucleic acid sequence is encoded by a 5-gene cassette containing precursor (XyeA), radical SAM enzyme (XyeB), protease (XyeC), transporter (XyeD), and fused protease transporter (XyeE). The radical SAM enzyme (XyeB) introduces the 3 rings and the protease-transporter (XyeE) cleaves the modified precursor. All genetic components to produce the antibiotic have been identified and functionally validated (substrate, enzymes, protease, and transporter). This opens up opportunities for applying these enzymes to modify non-cognate core peptide sequences, hence their relative flexibility in antibiotic discovery. This allows for a more efficient way of producing the natural products. The polypeptides are also stable to heat, proteolytic degradation, and low pH. The polypeptides may also be effective against Gram-negative bacteria, including clinical strains which are resistant to last-line antibiotics. Only a limited number of antibiotics have been approved that selectively target Gram-negative bacteria.

In contrast, Darobactin, which is the most comparable antibiotic is produced from by the dar gene cluster, contains 5 genes (precursor, radical SAM enzyme, and 3 x transporters). The radical SAM enzyme (DarE) is responsible for the 2-rings in the natural product. The protease responsible for cleavage has not been identified. To obtain the darobactin, an undefined protease in E coli is used.

Example 3: *xncAB* and *xncCDE*

For the production of xenorceptides, it was first established that **1** can be produced in

E. coli by expressing the xnc BGC split into two vectors: His_6-*xncAB* in pET28a(+) and *xncCDE* in pCDFDuet-1. The xncA gene was expressed with as an N-terminal His x 6 tag (His_6) so that the precursor could be purified, and the modifications detected (Figure 6). This two-vector system allows testing of His_6-*xyeAB* expressions first to ensure maturation by the rSAM/SPASM enzyme then *xyeCDE* in a second vector can be expressed in a subsequent expression to facilitate cleavage and export (Figures 3a and 3b). 3 BGCs named *smc*, *etc*, and *pac* from *Serratia marcescens*, *Erwinia toletana*, and *Photorhabdus australis*, respectively, were selected for heterologous expression (Figure 7).

To initiate heterologous expression, native AB constructs were synthesized and inserted into pET28a vector. The three constructs containing His_6-AB were expressed in E. coli NiCo21(DE3) cells. The precursors were purified by Ni-affinity chromatography, digested with trypsin and subjected to LC-MS. As demonstrated in Figure 3a, the digest obtained from the His6-SmcAB construct included a triply-charged fragment at m/z 903.7661, corresponding to -6 Da mass loss from the C-terminal region of SmcA (ALAQSMLDSVSGGWVNAFAR-WSKSF, m/z 905.7831 $[M+3H]^{3+}$). Expressions of His_6-EtcAB and His_6-PacAB constructs also resulted in detecting similar modified fragments (Figures 8 and 9). These experiments showed efficient modification by rSAM enzymes in E. coli and we proceeded with full cluster expression.

The remaining genes (CDE) for each cluster were synthesized and inserted into pCDFduet-1. Native His_6-XyeAB constructs were co-expressed with native XyeCDE constructs in E. coli. Both the cell biomass and the medium were analyzed separately by two methods. First, the cell pellet was processed as above to detect whether the precursor peptide was cleaved. Purified His_6-PacA, His_6-SmcA, and His_6-EtcA were detected as truncated leaders losing C-terminal residues after the GG motif, implying the protease is functioning (Figures 3b, 8, and 9). Second, the products were extracted from the culture medium using solid-phase extraction. The desired end products from smc, etc, and pac clusters were either undetectable or detectable in trace amounts. This result suggested D or E transporters are not functioning efficiently for native His_6-AB+CDE expressions (Figures 3b, 8, and 9). To increase the yields of end products, nonnative combinations of His_6-AB +CDE were tested. As shown in Figure 3c, Smc, Etc, and Pac products (**2-4**) could be efficiently produced using combinations of native His_6-XyeAB + XncCDE at a yield of 1.0-4.6 mg per liter. Tandem mass spectrometry (MSMS)

analysis of these products confirmed the primary amino acid sequence and localized -2 Da losses to each of the three Ω1-X2-X3 motifs.

Example 4: Characterisation

The structures of products **2-4** were characterized by NMR to understand whether the XyeB maturases from different Genera catalyze cyclophane formation with identical substitution pattern and the planar chirality with respect to the indole. Products **2-4** were characterized analogous to xenorceptide A1 reported previously. In all cases, the XyeB maturases carry out the same crosslinking of Trp as in **1** (Figure 4a). The Phe residue in **3** was assigned as para-substituted analogous to **1** (Figure 4b). However, **2** was elucidated as meta-substituted based on 2D NMR. Phe5-H2 (δ 6.91 ppm) appears as a singlet and has NOESY correlations with both Phe5-Hβb (δ 2.73 ppm) and Arg7-Hβ (δ 2.87 ppm). The remaining three aromatic protons within the same spin system (H4, δ 7.17 ppm; H5, δ 7.25 ppm; H6, δ 7.09 ppm) exhibit NOESY correlations with Phe5-Hβa (δ 2.96 ppm) and Arg7-Hγ (δ 2.10, δ 1.94 ppm), suggesting these protons lie on the same face and the new $C(sp^2)$-$C(sp^3)$ bond is formed between Phe5-C3 with Arg7-Cβ (Figure 4b). The Pac product (**4**) encodes a Tyr5 instead of Phe5, and the Tyr is crosslinked at C3 of Tyr (Figure 4b). This substitution pattern has been observed by triceptide maturases reported previously. The relative conformations of the cyclophane rings were assigned by NOESY and coupling constant analysis, which showed the orientation of the indole in the Trp-derived cyclophanes are identical for **1-4**. The absolute configuration of X2 residues were assigned by advanced Marfey's method in addition to guanidine isothiocyanate derivatization. These analyses led to all α-positions to be of the natural L-configuration and the remaining amino acids to be as shown. The planar chirality of the Trp was assigned as Sp. The Smc, Etc, and Pac products were named xenorceptide A2 (**2**), xenorceptide A3 (**3**), and xenorceptide A4 (**4**), respectively (Figure 4).

Structural eludication of xenorceptide A2 (**2**), xenorceptide A3 (**3**) and xenorceptide A4 (**4**) are shown in Figure 26-28. Figure 29-45 shows the NMR spectra used to derive the xenorceptide structures. Table 18-20 shows the summarised NMR data for these xenorceptides.

Example 5: Antibacterial activity

The four xenorceptides (**1-4**) along with unmodified sequences were screened for antibacterial activity. Minimal inhibitory concentrations (MICs) were obtained for **1-4** using microbroth dilution assays against Gram-positive and Gram-negative bacteria (Table 10). **2-4** showed selective activity against Gram-negative pathogens, E. coli ATCC 25922 and K. pneumoniae ATCC 700603 (Table 10). No activity was observed against Gram-positive bacteria (B. subtilis ATCC 6633 and S. aureus ATCC 29737) for any of the products tested. Encouraged by the activity of xenorceptide A2 (**2**) further testing was carried out on a broader panel including multi-drug resistant pathogens.

TABLE 9. MIC values (μg/mL) of xenorceptide A2 (**2**) against Enterobacteriaceae.

Species	Strain[a]	Xenorceptide A2 (**2**)
Escherichia coli	M6	8
	M10	4
	M11	4
	CRE1006	4
	ATCC 25922	4
Klebsiella pneumoniae	CRE 1007	8
	CRE1008	8
	CRE1011	8
	CRE1012	8
	ATCC 700603	8
Enterobacter cloacae	CRE1010	4
	CRE1014	16
	CRE1015	32
	CRE1016	16
	CRE1017	32
Salmonella typhimurium	ATCC 14028	8
Salmonella entereditis	ATCC 13076	8
Shigella flexneri	M90T	2

[a]CRE strains are carbapenem-resistant clinical isolates. M6, M10, and M11 strains are carbapenem- and colistin-resistant clinical isolates.

Table 10. Antimicrobial activity of **1**–**4**.

	MIC (μg/mL)				
Strain	Xenorceptide A1 (**1**)	xenorceptide A2 (**2**)	xenorceptide A3 (**3**)	xenorceptide A4 (**4**)	xenorceptide A8 (**8**)
Gram-negative bacteria					
Escherichia coli ATCC 25922	64	4	8	8	2
Klebsiella pneumoniae ATC 700603	64	8	8	16	4
Morganella morganii ATCC 25830	>64	32	64	64	-
Pseudomonas aeruginosa ATCC 9027	>64	64	64	>64	64
Acinetobacter baumanii ATCC 19606	>64	>64	>64	>64	>64
Gram-positive bacteria					
Bacillus subtilis ATCC 6633	>64	>64	>64	>64	-
Staphylococcus aureus ATCC 29737	>64	>64	>64	>64	>64

Table 11. MIC value of xenorceptide A2 (**2**) against bacterial pathogens.

Species	Strain	MIC (μg/mL)	Species	Strain	MIC (μg/mL)
Gram-negative bacteria (Enterobacteriaceae)			**Gram-negative bacteria (Other families)**		

Escherichia coli	M6	8	*Acinetobacter baumannii*	ACBA1001	32
	M10	4		ACBA1002	32
	M11	4		ACBA1003	32
	CRE1006	4		ACBA1004	64
	ATCC 25922	4		ATCC 19606	>64
Klebsiella pneumoniae	CRE 1007	8	*Pseudomonas aeruginosa*	DR4877/07	64
	CRE1008	8		DR5790/07	64
	CRE1011	8		DM4150R	64
	CRE1012	8		DM23376	>64
	ATCC 700603	8		ATCC 9027	64
Enterobacter cloacae	CRE1010	4	*Morganella morganii*	CRE1001	32
	CRE1014	16		ATCC 25830	32
	CRE1015	32			
	CRE1016	16	**Gram-positive bacteria**		
	CRE1017	32	*Staphylococcus aureus*	ATCC 29737	>64
Salmonella typhimurium	ATCC 14028	8		ATCC 43300	>64
Salmonella entereditis	ATCC 13076	8	*Bacillus cereus*	ATCC 11778	>64
Shigella flexneri	M90T	2	*Bacillus subtilis*	ATCC 6633	>64

Xenorceptide A2 (**2**) was tested against a larger panel of drug-resistant clinical isolates. These results are summarized in Table 9 and confirm the selective activity against Gram-negative Enterobacteriaceae, several of which are carbapenem-resistant Enterobacteriaceae (CRE) pathogens. Next, time-kill assays against the colistin-resistant strain E. coli M6 was carried out which showed that xenorceptide A2 (**2**) has a bactericidal effect over 24 h at both 4× and 8× MIC, causing 3-log reduction in bacteria

count (Figure 5a). To further understand the killing effect of xenorceptide A2 (**2**), we imaged the morphology of E. coli M6 in the presence of xenorceptide A2 (**2**) by scanning electron microscopy (Figure 5b). These images show significant disruption of the bacteria membranes within 2 h of treatment, followed by cell lysis and death (Figure 5b). Xenorceptide A2 (**2**) did not exhibit any cytotoxicity against HepG2 human cells up to a concentration of 256 µg/ml. Finally, we incubated xenorceptide A2 (**2**) at sub-inhibitory concentrations with E. coli M6 to test if resistance developed. Over the course of two weeks, we obtained strains that were ~4-fold resistant to xenorceptide A2 (**2**) with an MIC of 32 µg/ml (Figure 5c).

Example 6: Discussion

Natural products have been the main source of currently used antibiotics but no new classes of antibiotics have been introduced since the 1980s. Over the last few decades, bioactivity-guided isolation discovery has suffered from rediscovery of known compounds. The fundamental difference between the present invention and bioactivity-guided isolation is the former prioritizes chemistry while the latter prioritizes the bioactivity. In the present invention, only unknown molecules are screened, and MIC values are obtained directly. To the best of the inventors' knowledge, a natural product of a new chemotype able to selectively kill CRE pathogens has not been identified using a chemically-guided approach.

Using bioactivity-guided approaches, promising antibiotics against Gram-negative pathogens have been isolated from the entomopathogenic bacteria, Xenorhabdus and Photorhabdus. Odilorhabdins are broad spectrum peptide antibiotics that bind to a new ribosome site. Previous work has identified darobactin from strains of Photorhabdus by testing of concentrated extracts (20x). Recently, this concept was developed further to assay HPLC fractions of Xenorhabdus and Photorahbdus extracts representing a 200x fold increase in concentrations, which led to the antibiotic, 3'-amino-3'-deoxyguanosine, a pro-drug with selective activity against E. coli.

Structural similarities and differences are apparent in xenorceptide A2 and darobactin. The C-terminal pentapeptide of both share an identical Trp-derived cyclophane appended to Ser-Phe. Differences are in the N-terminus. Xenorceptide A2 has two three-residue cyclophanes separated by an Ala residue. Darobactin contains a second ether crosslinked cyclophane that is fused to a central Trp residue. Darobactin has broad

spectrum activity against Gram-negative pathogens and the mechanism of action was shown to bind to the bacterial insertase BamA20, an essential outer membrane protein in Gram-negative bacteria. Significantly, it is shown that xenorceptide A2 composed of non-fused three-residue cyclophanes has activity against specific Gram-negative bacteria. While the mechanism of action for xenorceptide A2 remains to be elucidated, the N-terminal cyclophanes appear to confer a greater selectivity for Enterobacteriaceae vs other bacteria.

In conclusion, GEnSyBER-A as an end to end workflow for the discovery of RiPP antibiotics is presented. This work-flow was applied to identify Xenorceptide A2 from radical SAM sequence function space. Xenorceptide A2 has promising activity against priority pathogens for which antibiotics are urgently needed. The strains of Serratia from which xenorceptide A2 is encoded are clinical isolates which may represent important and understudied sources for antibiotics.

Example 7: Bioinformatic mapping of xye BGCs

The Xye maturase systems encode a precursor (XyeA), rSAM/SPASM maturase (XyeB), protease (XyeC), transporter (XyeD), and protease/transporter (XyeE). The XyeA precursors are ~55 AA in length with the core sequences being typically 13-16 residues. Core peptides contain a ΩxxxΩxxΩxx motif (Ω1 = Trp, Phe or Tyr) where all Ω residues are involved in a 3-residue cyclophane. The Gly-Gly motif XyeA indicates the end of the leader sequence. In our bioinformatic analysis, we identified 81 XyeA precursors with 37 encoding unique core sequences (Table 3; Type A). The latter represents the total number of different xenorceptides that could be produced. In addition to the canonical type described above, three additional core types are readily identified based on homology to rSAM/SPASM XyeB maturases in the RefSeq database. The second, third, and fourth types contain ΩxxΩxx (Type B, n = 2 unique core sequences), ΩxxxΩxx (Type C, n = 1 unique core sequence), and ΩxxxxΩxx (Type D, n = 16 unique core sequences) motifs, respectively. We suggest that precursor types B-D are classified under xenorceptides (Table 3) because all precursors contain the Gly-Gly motif, BGCs typically conserve the characteristic five genes (xyeABCDE), and several maturases are identified by the cut-off defined for annotating XyeB radical SAM/SPASM proteins (TIGR04496) (Figure 10d). We predict that maturases from types B-D will also catalyze formation of triceptide macrocycles. The main source bacteria belong to the order Enterobacterales and a phylogentic tree based on the gene sequences for xyeB from

Type A precursors was constructed (Figure 11a). The 5 predominant genera that encode xye BGCs are Erwinia, Xenorhabdus, Serratia, Yersinia, and Photorhabdus. The source microbiomes of the bacteria are plants, nematode, and animals. Representative BGCs and core sequences from different genera are shown in Figure 11b. With bioinformatic mapping of the Xye maturase system complete, we proceeded to produce selected xenorceptides using synthetic biology.

Example 8: Heterologous expression of xenorceptides in E. coli.

For production of xenorceptides, we used two different expression systems that allowed systematic production of xenorceptides from different bacterial genera. We first established that **1** can be produced in E. coli by expressing the xnc BGC split into two vectors: His6-xncAB in pET28a(+) and xncCDE in pCDFDuet-1. The xncA gene was expressed with as an N-terminal His x 6 tag (His6) so that the precursor could be purified, and the modifications were detected (Figure 6). This two-vector system allows testing of His6-xyeAB expressions first to ensure maturation by the rSAM/SPASM enzyme then xyeCDE in a second vector can be expressed in a subsequent expression to facilitate cleavage and export (Figure 3a and 3b).

To initiate heterologous expression, native AB constructs were synthesized and inserted into pET28a(+) vector (Table 8). The three constructs containing His6-A+B were coexpressed in E. coli NiCo21(DE3) cells. The precursors were purified by Ni-affinity chromatography, digested with trypsin and subjected to LC-MS. As demonstrated in Figure 3a, the digest obtained from the smcAB construct included a double-charged fragment at m/z 1389.6797, corresponding to -6 Da mass loss from the C-terminal region of SmcA (ELVDSLLDTVSGGWVNAFARWSKSF, m/z 1392.7032 $[M+2H]^{2+}$). Expressions of etcAB and pacAB constructs also resulted in detecting similar modified fragments. These experiments showed efficient modification by rSAM enzymes in E. coli and we proceeded with full cluster expression.

The remaining genes (CDE) for each cluster were synthesized and inserted into pCDFduet-1. Native His6-A+B constructs were coexpressed with native XyeCDE constructs in E. coli Nico21(DE3). Both the cell biomass and the medium were analyzed separately by two methods. First, the cell pellet was processed as above to detect whether the precursor peptide was cleaved. Purified His6-SmcA, His6-EtcA, and His6-PacA were detected as truncated leaders losing C-terminal residues after the GG motif,

implying the protease (C or E) are functioning (Figure 3b). The products were extracted and purified from the culture medium by solid-phase extraction using a reversed-phase polymeric resin. The desired end products from *smc*, *etc*, and *pac* clusters were either undetectable or detectable in trace amounts (Figure 3b). This result suggested D or E transporters are not functioning efficiently for native His6-AB+CDE expressions. To increase the yields of end products, we tested nonnative combinations of His6-AB +CDE; i.e. AB is from one species and CDE is from another species. As shown in Figure 3c, Smc, Etc, and Pac products could be efficiently produced using combinations of native His6-XyeAB + XncCDE. In this case, XyeAB are selected from SmcAB, EtcAB and PacAB. Tandem mass spectrometry (MSMS) analysis of these products confirmed the primary amino acid sequence and localized -2 Da losses to each of the three Ω1-X2-X3 motifs. Using these combinations, we proceeded with production of the Smc, Etc, and Pac products by larger scale fermentation, solid-phase extraction (polymeric resin), and preparative reversed phase HPLC which provided sufficient material for biological testing.

The second approach used to produce xenorceptides was expression of chimeric leader-core hybrids with the Xnc maturation and export machinery. These constructs were composed of His6-XncA leader (His6-XncAL) fused to the XyeA core of the target natural product inserted in pET28a(+). This precursor construct was coexpressed with XncBCDE encoded in pCDFDuet-1. This combination of genetic components allows a small gene fragment for the precursor to be synthesized and avoids the costly synthesis of the transport machinery. Using these constructs we pursued production of the products from different bacterial genera including: Yersinia kristensenii (*ykc*), Xenorhabdus sp. (*xec*), Sodalis sp. (*soc*), Aeromonas jandaei (*ajc*), Provedencia huaxiensis (*phc*), and Vibrio sagamiensis (*vsc*) (Figure 12a and 12b). Upon fermentation and extraction all of these products could be detected and analyzed -2 Da mass losses localized to the expected motifs. However, the products from phc and vsc were not produced in sufficient amounts for biological evaluation. With suitable constructs in hand, we proceeded with larger scale production of **5-8** for biological evaluation.

Example 9: Antibacterial activity of xenorceptides.

The eight xenorceptides along with synthetic versions of the unmodified peptide sequences were screened for antibacterial activity. Our initial panel for testing consisted of quality control strains representing Gram-positive and Gram-negative bacteria (Table

10). Minimal inhibitory concentration (MIC) values were obtained for **1-8** using broth microdilution assays. While **1** showed weak or no activity, we were encouraged that **2-4, and 8** showed selective activity for Gram-negative pathogens (*E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603). No activity was observed against Gram-positive bacteria (*B. subtilis* ATCC 6633 and S. aureus ATCC 29737) for any of the products tested, and suggests the bioactive products are selective against Gram-negative strains. The unmodified synthetic peptides representing the core sequences from **2-4** also did not show any bioactivity against Gram-negative and Gram-positive bacteria, which confirms that the cyclophane rings are critical to the bioactivity of the Xye peptides. Encouraged by the activity exhibited by **2-4**, we carried out structure elucidation and further biological evaluation.

Example 10: Structure elucidation of xenorceptides.

The structures of products **2-4** were characterized by NMR spectroscopy to understand whether the XyeB maturases from different genera catalyze cyclophane formation with identical substitution pattern and the planar chirality with respect to the indole, using NMR spectra, assigned chemical shifts, and key correlations. Products **2-4** were characterized analogous to xenorceptide A1. In all cases, the XyeB maturases carry out the same crosslinking of Trp as in **1** (Figure 4a). The Phe residue in **3** was assigned as para-substituted analogous to **1** (Figure 4b). However, **2** was elucidated as meta-substituted based on 2D NMR. Phe5-H2 (δ 6.91 ppm) appears as a singlet and has NOESY correlations with both Phe5-Hβb (δ 2.73 ppm) and Arg7-Hβ 195 (δ 2.87 ppm). The remaining three aromatic protons within the same spin system (H4, δ 7.17 ppm; H5, δ 7.25 ppm; H6, δ 7.09 ppm) exhibit NOESY correlations with Phe5-Hβa (δ 2.96 ppm) and Arg7-Hβ (δ 2.10, δ 1.94 ppm), suggesting these protons lie on the same face and the new C(sp2)-C(sp3) bond is formed between Phe5-C3 with Arg7-Cγ (Figure 4). The Pac product (**4**) encodes a Tyr5 instead of Phe5, and the Tyr is crosslinked at C3 of Tyr (Figure 4). This substitution pattern has been observed by triceptide maturases reported previously. The relative conformations of the cyclophane rings were assigned by NOESY and coupling constant analysis, which showed the orientation of the indole in the Trp-derived cyclophanes are identical for **1-4**. The absolute configuration of X2 residues were assigned by advanced Marfey's method in addition to guanidine isothiocyanate derivatization. These analyses led to all α-positions to be of the natural L-configuration and the remaining amino acids to be as shown. The planar chirality of

the Trp was assigned as Sp. The Smc, Etc, and Pac products were named xenorceptide A2 (**2**), xenorceptide A3 (**3**), and xenorceptide A4 (**4**), respectively (Figure 4).

Structural eludication of xenorceptide A2 (**2**), xenorceptide A3 (**3**) and xenorceptide A4 (**4**) are shown in Figure 26-28. Figure 29-45 shows the NMR spectra used to derive the xenorceptide structures. Table 18-20 shows the summarised NMR data for these xenorceptides.

Example 11: Biological evaluation of xenorceptide A2.

Xenorceptide A2 (**2**) was tested against a larger panel of clinical drug-resistant isolates. These results are summarized in Table 11 and confirm the selective activity (2-8 g/ml MICs) against Gram-negative Enterobacteriaceae, several of which are carbapenem-resistant Enterobacterales (CRE) pathogens. Next, we carried out time-kill assays against E. coli M6 (a carbapenem- and colistin-resistant clinical isolate) which showed that xenorceptide A2 (**2**) has a bactericidal effect over 24 h at 8 x MIC, causing 3-log reduction in bacteria count (Figure 13a). To further understand the killing effect of xenorceptide A2 (**2**), we imaged the morphology of E. coli M6 in the presence of xenorceptide A2 (**2**) by scanning electron microscopy. Within 4 h of peptide treatment, the cells showed clear membrane damage and surface blebbing, followed by cell lysis and death (Figure 13c). Xenorceptide A2 did not show any cytotoxicity against HepG2 human cells up to a concentration of 256 µg/ml. To understand resistance development, we incubated xenorceptide A2 at sub-inhibitory concentrations with E. 221 coli M6. Over the course of two weeks we obtained strains that were ~4-fold resistant to xenorceptide A2 (**2**) with an MIC of 32 µg/ml (Figure 13b). In contrast, E. coli M6 readily became less susceptible to colistin at an earlier time point than xenorceptide A2 (**2**). After extensive in vitro biological evaluations, we evaluated the in vivo antimicrobial efficacy of xenorceptide A2 (**2**) using a peritonitis model in neutropenic mice (Figure 13d). After 30 min of inoculation with E. coli M6, mice (n = 5 per group) were given a single intraperitoneal injection of treatment or saline. At 5 h post-treatment, the mice were euthanized for collection of peritoneal fluid, blood, and organs for quantification of bacteria burden using colony counting method. Xenorceptide A2 (**2**) displayed concentration-dependent antimicrobial effect in peritoneal fluid, blood, and liver where 50 mg/kg dose caused a 6-, 7-, and 4-log decrease in colony count relative to saline control results, respectively (Figure 13e). While weaker effect was observed in spleen and kidney, 50 mg/kg xenorceptide A2 (**2**) still achieved 2-log reduction in bacteria

burden. At the same dose of 5 mg/kg, the peptide displayed comparable efficacy to colistin.

Example 12: Discussion

Antibiotics against Gram-negative pathogens are urgently needed. Natural products have been the main source of currently used antibiotics but no new classes of antibiotics have been introduced since the 1980s. Of the bacterial pathogens, Gram-negative are challenging for antibiotic discovery due to their dual membrane envelope. At current, there are two approaches for identifying natural product derived antibiotics. The first is using bioactivity-guided isolation. These platforms typically start with in vitro cell based assays where activity from a crude or partially purified extract is prioritized. A series of purification and retesting steps are carried out until the active component is isolated and characterized. This process was and remains the key process for which antibiotics have been discovered. However, over the last few decades, bioactivity-guided isolation discovery has suffered from rediscovery of known compounds. The second method is by producing targeted products directly for their chemical novelty – a chemically guided or chemistry first approach. The novelty may vary from as little as a functional group (congener of a known natural product) or could be a new and unpredictable scaffold. In this approach, the natural products are obtained by heterologous expression, host organism (native or engineered), or by chemical synthesis. We demonstrate the second approach to yield the targeted compounds directly and MIC values were obtained for each molecule produced.

In recent years promising antibiotics against Gram-negative pathogens have been described using bioactivity-guided approaches by exploiting unique bacterial sources, in particular the entomopathogenic bacteria, Xenorhabdus and Photorhabdus. While these organisms have been studied for their natural products, several antibiotics that target Gram-negative pathogens have been reported in recent years. Using a combination of different strategies (culturing under various conditions, co-culturing with other microorganisms, and mutations to the host RNA polymerase) led to the identification of odilorhabdins, broad spectrum peptide antibiotics from Xenorhabdus and Photorhabdus. In a separate study, darobactin was identified from strains of Photorhabdus by testing of 20x concentrated extracts. This concept was developed further to assay HPLC fractions representing 200x fold increase in concentrations, which led to the antibiotic, 3'-amino-3'-deoxyguanosine, a pro-drug with selective activity against E. coli and

dynobactin, a second RiPP natural product able to target Gram-negative bacteria by inhibition of BamA.

Genome mining and synthetic biology have reinvigorated drug discovery from natural products and enabled chemistry-first approaches to advance. However, the discovery of selective inhibitors of Gram-negative bacteria using this approach has been less successful. One drawback is the need to treat each BGC on a case-by-case basis and requires specific manipulation for heterologous expression or activation of the pathway in host strains. We addressed some of these difficulties by developing two systems to access several natural products from different BGCs. Another approach independent of a producing microorganism has been to chemically synthesis natural products directly based on BGC-predicted compounds. This has been demonstrated by Wang and coworkers to identify macolacins, that show promising activity against Gram-negative bacteria. This methodology is most suited when the structures can be accurately predicted and the natural products are amenable to synthesis. For xenorceptide A2, bioinformatic prediction would have predicted the para-substituted Phe-derived cyclophane possibly resulting in a less or inactive product. The recent total synthesis of darobactin demonstrates the difficulty and complexity of synthesizing this class of molecules and represents a significant challenge. In this scenario, heterologous production has clear advantages over other methods for production.

Another potential drawback of chemistry first approaches is that the bioactivity of the target compounds cannot be predicted with certainty. However, some clues to what bioactivity can be expected using the composition of the BGC as a rudimentary guide. In this example, xye BGCs are reminiscent of microcin or bacteriocin BGCs so we suspected the products may contain bactericidal activity. During the course of our work, the discovery of darobactins and dynobactins supported that xenorceptides possessing antibiotic activity likely existed. We proved our hypothesis to be valid for selected products obtained. This result was encouraging and supports that further production and testing of the remaining genetically encoded xenorceptides or variants may lead to products with higher potency, selectivity for other pathogenic bacteria, or have broader spectrum activity.

The C-terminal pentapeptide of xenorceptide A2 (**2**) including the 3-residue cyclophane is identical in sequence and configuration compared to darobactin. Darobactin has broad

spectrum activity against Gram-negative pathogens and the mechanism of action was shown to bind to the bacterial insertase BamA, an essential outer membrane protein in Gram-negative bacteria. The N-terminus of xenorceptide A2 carries two distinct three-residue cyclophanes separated by a single amino acid. This feature differentiates xenorceptide A2 from both daroactin and dynobactin. Of significance with regard to the structures of dynobactin and xenorceptide A2 is that non-fused three-residue cyclophanes are able to inhibit selected Gram-negative bacteria. Xenorceptide A2 is more potent than dynobactin and has comparable potency to darobactin against Enterobactericeae. Another notable effect for xenorceptide A2 is that resistance development halted at 4xMIC and occurred over a period of 6-8 days. This shows that E. coli are less resistant to xenorceptide A2 compared to darobactin. While the mode of action for xenorceptide A2 remains to be elucidated, the two N-terminal cyclophanes appear to confer a greater selectivity for specific genera within Enterobacteriaceae. The producers of xenorceptides A2 (Serratia species) and G (Aeromonas jandaei) that have the highest potency against Gram-negative bacteria are derived from human samples while the other host strains are from other animals or plants.

RiPP cyclophanes are among the most promising chemotypes for antibiotic development against Gram-negative pathogens. Their advantages include resistance to proteases, water solubility, first in class potential, and possess a unique mode of action. The discovery of darobactin, dynobactin, and xenorceptides also demonstrate efficacy of the two existing techniques to identify natural product antibiotics. Darobactins and dynobactins were identified using host strains and innovative bioactive guided fractionation. The discovery of xenorceptide A was identified by producing a series within a natural product class then screening for activity. We used synthetic genes and cross-combinations of genetic components (hybrid BGCs) to enable the production of the desired natural products. We envisage a similar or optimized approach using different combinations of genetic components will allow access to the remaining xenorceptides. The systematic production and testing of natural product families will hopefully become more routine to identify new and potent antibiotics to control antibiotic resistance pathogens.

Example 13: Heterologous expression of xenorceptides A11 (**11**), A12-1 (**12**) and A12-2 (**13**) in *E. coli*.

For the production of xenorceptides A11 (**11**), A12-1 (**12**) and A12-2 (**13**), they were produced in *E. coli* by expressing the Smc2A/pET28a(+), Smc3A-1/pET28a(+) or Smc3A-2/pET28a(+) + Smc3B-XncCDE/pCDFDuet-1. The Smc2A, Smc3A-1 or Smc3A-2 gene was expressed as an N-terminal His x 6 tag (His_6) so that the precursor could be purified, and the modifications detected (Figures 14-16). This two-vector system allows His_6-*xyeA* precursor peptides modified by the rSAM/SPASM enzyme *xyeB* followed by *xncCDE* to cleave and export that is in a similar manner as above mentioned xenorceptides (Figures 3a and 3b).

The His_6-Smc2A/pET28a(+), His_6-Smc3A-1/pET28a(+) or His_6-Smc3A-2/pET28a(+) construct was co-expressed with Smc3B-XncCDE/pCDFDuet-1 construct in *E. coli*. The cell medium was analyzed by extraction of the culture medium using solid-phase extraction (SPE). The desired end products, xenorceptide A11 (**11**), xenorceptide A12-1 (**12**) and xenorceptide A12-2 (**13**) from Smc2A, Smc3A-1 and Smc3A-2 precursors, respectively were detected from LCMS and confirmed by MSMS analysis to localized -2 Da losses to each of the three Ω1-X2-X3 motifs (Figures 14-16). To sufficiently produce the end products **11-13** for antimicrobial assays, large scale culture was carried out. Total 10 liter of Smc2A, 6 liter of Smc3A-1 and 8 liter of Smc3A-2 were cultured, SPE extracted and HPLC purified to yield **11** (8.5 mg, 0.85 mg per liter), **11** (3.6 mg, 0.60 mg per liter) and **11** (5.5 mg, 0.68 mg per liter). Xenorceptide A11 (**11**), xenorceptide A12-1 (**12**) and xenorceptide A12-2 (**13**) were tested against a panel of clinical drug-resistant isolates. These results are summarized in Table 15.

Example 14: Full cluster expression of type B and type D xenorceptides

The Xye maturase system (GenProp1090) is derived from the names of three bacterial genera where it is commonly found: Xenorhabdus, Yersinia, and Erwinia. The substrate precursors are collectively referred to as XyeA, the rSAM proteins as XyeB, the proteases as XyeC, the transporters as XyeD, and the proteases/transporters as XyeE. Type B XyeA precursors containing ΩxxΩxxxx (n = 2) and type D precursors containing ΩxxxxΩxxxx (n = 16) through homology searches of rSAM/SPASM XyeB maturases in the RefSeq database. Subsequently, we screened the function of all the rSAM through co-expression of the precursor-rSAM pairs in *E. coli*. Based on these screening results, we have selected certain type B and type D family BGCs for full-gene cluster expression, specifically *xgc*, *psc*, *poc*, *phc*, *kcc2*, *bbc*, *kcc1* and *plc* (as shown in Figure 17). These three-letter short name to the gene clusters were given from the strain *Xenorhabdus*

griffiniae VH1 (*xgc*), *Pandoraea* sp. PE-S2R-1 (psc), *Pandoraea oxalativorans* DSM 23570 (*pol*), *Photorhabdus heterorhabditis* Q614 (*phc*), *Kosakonia cowanii* pasteuri (*kcc2* and *kcc1*), *Bordetella bronchialis* AU17976 (*bbc*) and *Photorhabdus laumondii* BOJ-47 (*plc*). For the *xgc* cluster, which contains two precursor genes, we named these two precursors XgcA1 and XgcA2. Additionally, the *kcc2* and *kcc1* clusters share the same protease and transporter, so both *kcc2AB* and *kcc1AB* were coexpressed with the protease and transporter genes labeled *kcc2CDE*.

To investigate whether XyeCDE can function on corresponding Xye precursor in *E. coli*, type B and type D family His6-tagged precursor and rSAM genes constructs were synthesized and inserted into pRSFDuet-1 vector, along with the relevant protease, transporter genes were cloned onto pCDFDuet-1 vector. These pairs of plasmids were then transformed into E. coli NiCo (DE3) host cells. The two-vector system enables testing of His6-*xyeAB* expression to ensure proper maturation by the rSAM enzyme, followed by expression of *xyeCDE* in a second vector to facilitate cleavage and export.

Each gene cluster was fermented in a small scale of 200 mL in LB media firstly, then the truncated leader and modified full-length peptides were purified using Nickel-affinity chromatography and digested with trypsin; the end products were purified by solid phase extraction (SPE) from culture media. The full-length peptides, truncated precursors, trypsin digested fragments and end products were then detected through LC-MS analysis.

Similarly, genes of each cluster's His6-tagged precursor and rSAM enzyme were cloned into pRSFDuet-1 plasmid, while the relevant protease, transporter genes were cloned into pCDFDuet-1 plasmid. These pairs of plasmids were then transformed into E. coli NiCo21 host cells. The two-vector system enables testing of His6-*xyeAB* expression to ensure proper maturation by the rSAM/SPASM enzyme, followed by expression of xyeCDE in a second vector to facilitate cleavage and export. Each gene cluster was fermented in a small scale of 200 mL, then the full-length precursors were purified by nickel affinity chromatography, digested with trypsin and subjected to LCMS, the end products were purified by SPE form culture media.

Table 12. Summary of Xye Type B and Type D full-cluster expression screening

BGC	Core sequence	SEQ ID	Detection by LC-MS	
			Truncated Leader	Modified Core
xgcA1	ASTAET**WFK**LD**WKK**SF	54	Yes	Yes
xgcA2	SSDDDGI**FFK**TT**WDR**R	55	Yes	Yes
kcc2	RGEG**WVR**AY**WAK**RF	50	Yes	Yes
kcc1	DGR**WLQWIK**NH	41	Yes	Yes
phc	KPGEG**WVN**FT**WNK**SF	52	Yes	Yes
plc	GDR**WLKWIK**NH	40	Yes	No
poc	NV**FVN**AT**WSR**AM	47	No	No
psc	GNA**FVN**AT**WSR**AM	234	No	No
bbc	**FAN**AT**WSK**SF	233	No	No

The clear peaks of truncated leaders from LC-MS data suggested that protease from *xgc*, *phc*, *kcc2* and *phc* clusters can work well in E. coli for their corresponding precursors, and the cleavage site of these cluster are the GG motif as predicted. In the precursors XgcA1, XgcA2 and PhcA, there is an arginine located at the C-terminal immediately adjacent to Gly-Gly, which serves as the cleavage site of trypsin. Therefore, only full-length data for these three precursors are presented. (Figure 18) Taking XgcA1 as an example, the LC-MS data shows that both mono-modified (-2D) and bi-modified (-4D) full-length precursors can be detected in both XgcA1B and XgcA1B + XgcDEC expression systems. However, the truncated leader that cleaves at the GG motif is only present in the full-cluster expression system. This suggests that the presence of protease is necessary for the successful cleavage of the XgcA1 precursor at the Gly-Gly motif. (Figure 18)

In the case of *kcc2* and *kcc1*, truncated leader is detectable in full-length, but in small quantities, so only the relatively clear digested fragment is shown. The characteristic fragment "AAHVANLLDNVQGG" ($[M+H]^+$, m/z 1378.3395) is only detectable in Kcc2AB + Kcc2CDE expression, and similarly characteristic fragment "FSQSLLDDVQGG" ($[M+H]^+$, m/z 1151.5164) " is only detectable in *kcc1* full-cluster expression.

Observations have revealed that the *plc* precursor contains three consecutive Gly motifs at its C-terminal. (Figure 19a) In full-length LCMS samples, significantly truncated precursors were detected from the first two GG motifs, (Figure 19b, c) and similarly, trypsin-digested samples also showed clear evidence of cleavage at the first two GG motifs in the Plc precursors, supporting that these motifs act as a cleavage site. However, no product was detected in the supernatant, which suggests that the plc protease can function in *E. coli*, but the transporter is not operational in this organism. (Figure 19). The other three clusters *psc*, *bbc* and *poc*, we attempted to use various combinations of proteases and transporters, but no desired compound was detected. Alternative strategy would be utilized on these clusters.

LC-MS data from small-scale SPE experiments revealed that full gene cluster expression of *kcc2*, *kcc1*, *phc*, *xgc* (A1 and A2) led to the detection of their respective end products, as compared to only His6-XyeAB expression. As demonstrated in Figure 21, the products obtained from the *kcc2AB* + *kcc2CDE* construct included a double-charged fragment at m/z 889.4837, corresponding to -4 Da mass loss from the C-terminal core region of Kcc2A (RGEGWVRAYWAKRF, m/z 891.4710 $[M+2H]^{2+}$), as well as a double-charged fragment at m/z 890.4916, corresponding to -2 Da mass loss of the core fragment, and an unmodified fragment at m/z 891.4988. Similarly, expression of *kcc1* constructs resulted in the detection of -4 Da and -2 Da mass losses modified and unmodified core peptide fragments, which were displayed using an extracted ion chromatogram (EIC) in Figure 10c because they were trace amounts. Tandem mass spectrometry (MS/MS) was conducted to locate the modifications to specific residues. MSMS analysis localized the -2 Da modifications to the first Ω1X2X3 motif for Kcc2A core peptide and the second Ω1X2X3 motif for -2 Da Kcc1 product. For *phc* and *xgc* (A1and A2), only fully modified end products were detected. In comparing the precursor A1 and A2 of Xgc, the efficiency of the Xgc transporter for XgcA1 is higher than that for XgcA2, evidenced by the significantly larger amount of XgcA1 end product detected in the supernatant compared to XgcA2. These results are summarized in Table 14 and illustrated in Figure 20-22.

Large scale fermentation followed by SPE and preparative reversed phase HPLC was carried out for *xgc(A1)*, *phc* and *kcc2* clusters based on their good yield in small-scale experiments, to obtain a sufficient amount of compound from *xgcA1*, *kcc2*, *kcc1*, *phc*, *plc*. However, the yields of compounds from *xgcA2*, *poc*, *psc* and *bbc* were relatively low, making it difficult to obtain sufficient quantities for biological evaluation by SPE.

Therefore, we designed several variants and utilize alternative strategies for *xgcA2* and *kcc1*, as well those clusters that failed in full cluster expression.

Example 15. In vitro cleavage of leader peptide from modified precursors

For the precursors that cannot be produced using the full-cluster expression strategy, we designed G-to-K/R/E variants in an attempt to obtain the predicted natural products via peptidase digestion. The core peptides are composed of 10–16 amino acids, which we have labelled with positive numbers starting from the first residue of the predicted core sequence. We were initially interested in the *bbc* cluster due to the presence of two Gly-Gly motifs at the C-terminal region (Figure 17), with the GG closer to the C-terminal adjacent to the first Ω, which is a unique feature of type A Xye precursors. However, it was found that the rSAM BbcB can only catalyze the formation of one ring, which different from previous screening results. To determine which GG motif is the boundary between leader and core peptide and investigate the possibility of using another rSAM to form two rings, we designed a fusion precursor consisting of the BbcA leader and Kcc2A core and co-expressed it with BbcB. The purified product was trypsin-digested and analyzed via LCMS, revealing that only the longer leader helped to produce -2D modification in the Kcc2A core. These results suggest that the boundary between the precursor and core is located at the second GG motif.

We investigated whether PocB rSAM could assist BbcA in forming two rings, as PocB has a high conversion rate to modify PocA, and the PocA core peptide is similar to the BbcA core. We also designed the Gly(-1) to Lys variant of PocA leader to generate the expected BbcA core peptide after trypsin cleavage. The results showed that PocB could indeed assist in the production of -4D and -2D modified BbcA core peptides, labelled compound **30** and **31**, respectively. (Figure 23c) We also designed variants of XgcA2(G-1K), Kcc1A(G-1E), and PocA(G-1R) to co-express their corresponding rSAM and then digested with appropriate peptidases to produce the predicted natural products. Figure 23 a, b, d shows that the yield of these targeted fragments was good. The core peptides of PlcA and PscA have similarities with Kcc1A and PocA, respectively.

After the large-scale fermentation of 14-18 L of each variant, nickel affinity chromatography was used for purification, followed by semi-preparative HPLC to obtain a certain amount of compound **22, 27, 28, 30 and 31**.

Table 13. Xye Type B and Type D core peptides

Compound	Sequence
21	ASTAET**W**FKLD**W**KKSF (SEQ ID 54)
22	SSDDDGI**F**FKTT**W**DRR (SEQ ID 55)
23	KPGEG**W**VNFT**W**NKSF (SEQ ID 52)
24	RGEG**W**VRAY**W**AKRF (SEQ ID 50)
25	RGEG**W**VRAYWAKRF (SEQ ID 50)
26	RGEGWVRAYWAKRF (SEQ ID 50)
27	DGR**W**LQ**W**IKNH (SEQ ID 41)
28	DGRWLQ**W**IKNH (SEQ ID 41)
29	DGRWLQWIKNH (SEQ ID 41)
30	**F**ANAT**W**SKSF (SEQ ID 233)
31	FANAT**W**SKSF (SEQ ID 233)
32	NV**F**VNAT**W**SRAM (SEQ ID 47)
33	NV**F**VNAT**W**SRAM (SEQ ID 47)

* Bold residues refer to X_1 of the three-amino acid motif, where a cyclophane is formed between X_1 and X_3.

Example 16. Antibacterial activity

To assess the antibacterial activity of the compounds under investigation and determine their minimum inhibitory concentration (MIC), we purchased linear core peptides as internal standards and employed a spectroscopic method to quantify the samples for preliminary screening. Promising compounds will be produced in larger quantities and subjected to a more accurate MIC measurement. Our panel for testing consisted of E. coli, K. pneumoniae, E. cloacae, A. baumannii, E. faecalis and S. aureus (Table 14). MIC values were obtained for the compounds **21-29** and **30**, **31**, using broth microdilution assays. XgcA1 (2**1**), XgcA2 (2**2**), and both -4D and -2D Bbc products (**30** and **31**) showed no activity against all the strains that we tested. But we were encouraged by Kcc2 (**24-25**), Phc (**23**) and Kcc1 (**27**), **27** only had selective activity against *K. pneumoniae* with MIC value 8 μg/mL, **23** had some activity against *E. coli*, *E. cloacae*, *A. baumannii* and *K. pneumoniae*, with MIC value range from 8-32 μg/mL. Notably, fully modified kcc2 core peptide (**24**) showed reasonable activity against Gram-negative strains E. coli, *E. cloacae*, *A. baumannii*, and *K. pneumoniae* with MIC value range from 1-4 μg/mL. From this result, it seems that the antibacterial activity of **24** is stronger but more narrow-spectrum than Darobactin, and selectively kills Gram-negative

bacteria. Secondly, **25**, which is single modified Kcc2 product, was also active against these test bacteria, but weaker than **24** that is fully modified, the unmodified product **26** was not active against any of the test bacteria, which confirms that the cyclophane rings are critical to the bioactivity of the Xye peptides.

Table 14. Antimicrobial activity

	MIC (μg/mL)										
Strain	21	22	23	24	25	26	27	28	29	30	31
Gram-negative bacteria											
Escherichia coli ATCC 25922	>64	>64	16	1	8	>64	>64	-	>64	>64	>64
Klebsiella pneumoniae ATC 700603	>64	>64	32	2	16	>64	8	-	>64	>64	>64
Enterobacter cloacae	>64	>64	32	4	16	>64	>64	-	>64	>64	>64
Acinetobacter baumanii ATCC 19606	>64	>64	64	2	16	>64	>64	-	>64	>64	>64
Gram-positive bacteria											
Enterococcus faecalis	>64	>64	>64	64	>64	>64	>64	-	>64	>64	>64
Staphylococcus aureus ATCC 29737	>64	>64	>64	>64	>64	>64	>64	-	>64	>64	>64

Table 15. MIC value of xenorceptides A11, A12-1, A12-2, D1 and B1 against bacterial pathogens

		Xenorceptide				
Strain	**Subtype**	**A11**	**A12-1**	**A12-2**	**D1**	**B1**
Escherichia coli	M2	8	8	4	4	>32
	M6	4	2	2	2	>32
	M10	2	2	2	2	>32
	M11	4	2	4	2	>32
	CRE1006	4	2	2	2	>32
	ATCC 25922	1	2	1	1	>32
Klebsiella pneumoniae	CRE 1007	4	2	4	4	>32
	CRE1008	4	4	4	4	>32
	CRE1011	4	4	8	2	>32
	CRE1012	4	4	4	4	>32
	ATCC 700603	-	-	-	2	-
Pseudomonas aeruginosa	DR4877/07	32	32	32	16	>32
	DR5790/07	32	32	32	16	>32
	DM4150R	16	32	32	32	>32
	DM23376	16	>32	32	16	>32
Acinetobacter baumanii	ACBA1001	16	8	16	4	>32
	ACBA1002	16	8	8	4	>32

Enterobacter cloacae	ACBA1003	16	8	16	4	>32
	ACBA1004	16	8	16	4	>32
	ATCC 19606	-	-	-	2	>32
	CRE1010	4	2	2	4	>32
	CRE1014	8	8	32	8	>32
	CRE1015	16	16	16	8	>32
	CRE1016	8	8	16	8	>32
	CRE1017	16	16	32	8	>32
	ATCC 13047	-	-	-	4	>32

Xenorceptide D1: SEQ ID 50; Xenorceptide B1: SEQ ID 40

Example 17. Structure elucidation

Compound **24** has the strongest and broadest spectrum of anti-microbial activity among all the type A, type B and type D xenorceptides we have obtained so far, so we decided to prioritize the production of sufficient amounts of **24** for structure analysis. Concentrated SPE elute fraction from 40 L culture of Kcc2AB coexpressed with Kcc2CDE was subjected to reverse phase preparative HPLC using a C18 column followed by a Luna PFP column to get ~6.8 mg of pure product.

Compound **24** is composed of 14 amino acids, which we have labelled with positive numbers starting from the first residue of the predicted core sequence (Figure 24). Sequential assignment of backbone NHs and their corresponding spin systems was performed using MS/MS and 2D NMR analysis, which confirmed the N-terminal (RGEG) and C-terminal (RF) sequences were unmodified. MS/MS of compound **24** showed −2 Da mass shifts localized to each of the WVR and WAK motifs within the predicted core peptide fragmentation, indicating that cyclization may have occurred within the two motifs.

Chemical shifts of side chain protons were assigned using COSY and TOSCY spectra. COSY and TOCSY correlations were observed between Hα and methyl group (Ala8 and Ala11) and through the spin system of iso-propyl side chain of Val6. The chemical shifts of Hβ/Cβ of Arg7 (δ 2.82 ppm/46.38 ppm) and Lys12 (δ 2.70 ppm/49.60 ppm) were assigned by TOCSY, COSY, and HSQC correlations starting from NH signals. 1H and 13C chemical shifts of the Trp5 and Trp10 were assigned starting from Arg7 Hβ/Cβ and Lys12 Hβ/Cβ respectively.

For the first macrocyclic ring, 2D NMR analysis indicated that Trp5 was now substituted at Trp5-C6, based on the following observations: Trp5-H4 (δ 7.15 ppm) and Trp5-H5 (δ 6.72 ppm) were assigned adjacent based on 3JHH coupling. The location of Trp5-H5 was supported by HMBC correlations to Arg7Cβ and a NOESY correlation to Arg7Hβ, 1H signals of Trp5-H5 appeared as a doublet. Trp5-H7 (δ 7.14 ppm) was assigned based on HMBC correlations to Arg7Cβ, a NOESY correlation to Arg7Hβ, Arg7Hγ (δ 2.13 ppm) and Trp5-indole NH (δ 10.74 ppm). The assignment of Trp5-H2 (δ 7.14 ppm) was supported by 3JHH coupling with Trp5-indole NH and a NOESY correlation to Trp5Hβ (δ 2.94 ppm). The indole NH gave correlations to C2, C3, C7, C7a. The protons for H1, H2, H4, H5, and H7 of Trp10 could be assigned while H6 was not observed. Collectively, these observations supported a new C-C bond between Trp5C6 and Arg7Cβ. Determination of the newly formed bond in the WAK motif was carried out in a similar fashion. Figure 25 revealed key correlations that allowed assignment of the newly formed bonds.

Figure 46-51 shows the NMR spectra used to derive the structure of xenorceptide D1 (**24**). Table 21 shows the summarised NMR data for xenorceptide D1 (**24**).

Materials, equipment, and general experimental procedures. Chemicals and reagents were purchased from the following suppliers: Acetonitrile from Tedia (USA); Isopropanol and methanol from Thermo Fisher Scientific (USA); Kanamycin and spectinomycin from GoldBio; Isopropyl β-D-1-thiogalactopyranoside (IPTG) from Combi-Blocks; and Strata-X® Polymeric Solid Phase Extraction (SPE) Sorbent (33 µm) from Phenomenex (USA); NMR solvent DMSO-d_6 from Cambridge Isotope Labs (USA). Other chemicals and reagents were purchased from either Sigma (USA) or Bio Basic (Canada). Synthetic genes inserted into expression vectors were purchased from Twist Bioscience (USA). *Escherichia coli* NiCo21(DE3) cells were purchased from New England Biolabs (USA). Electroporation was carried out using mode p2 (2.5 kV, 5.6 ms) on a MicroPulser Electroporator (Bio-Rad, USA). Ultrasonication was carried out using an Ultrasonic Cleaner 142-0307 (VWR, USA). Centrifugation was carried out using either an Eppendorf® Centrifuge 5424R or 5810R (Germany), or an Avanti JXN-26 Ultracentrifuge (Beckman Coulter, USA). SPE was performed using either 12-Position Vacuum Manifold Set (Phenomenex, USA) or Vac-Man® Vacuum Manifold (Promega, USA). Sample solutions were concentrated using either a rotary evaporator (Rotavapor® R-210, Büchi, Switzerland), centrifugal evaporator (Genevac EZ-2 Elite, SP Scientific,

UK), or freeze dryer (ScanVac CoolSafe, LaboGene, Denmark). LC-MS experiments were performed on a Waters Acquity UPLC System coupled to Xevo G1 QToF Mass Spectrometer (USA) and data was analyzed using MassLynx v.4.1. Preparative HPLC was carried out on a Shimadzu Nexera Prep System. NMR spectra were acquired at 298 K using a Bruker 400 MHz Avance Neo Nanobay NMR Spectrometer (USA) with a Bruker iProbe 5 mm SmartProbe or a Bruker 800 MHz Avance Neo NMR Spectrometer (USA) with a Bruker 5 mm CPTXI Cryoprobe and data was analyzed using Bruker Topspin v3.6.

Transformation of plasmids into *E. coli* cells. Plasmids containing precursor (*xyeA*) and rSAM (*xyeB*) genes or those containing peptidase and transporter (*xyeCDE*) genes were synthesized by Twist Bioscience. The plasmids were reconstituted in autoclaved Milli-Q grade 1 water to a final concentration of 10 ng/μL. For full-length gene cluster expression,1 μL of plasmid DNA was added to 70 μL of *E. coli* electrocompetent cells and transformed in a 2 mm electroporation cuvette. For coexpression, 1 μL of each plasmid DNA containing the appropriate genes was added to 70 μL of *E. coli* electrocompetent cells and transformed in a 2 mm electroporation cuvette. 1 mL of lysogeny broth (LB) was subsequently added to the transformed cells in an Eppendorf tube and incubated in the shaker at 37 °C, 200 rpm for 1 h. Following this, the bacteria cells were centrifuged at 4,000 rpm for 10 min at 25 °C and the cell pellet obtained by disposing the supernatant. The cell pellet was then resuspended with the residual supernatant and streaked on LB agar supplemented with appropriate antibiotics to be grown overnight at 37 °C.

Expression and purification of His_6-precursors. An overnight culture of the transformant was inoculated into LB medium in an Ultra Yield® flask (Thomson) at a ratio of 1:100 v/v with appropriate antibiotics. The flask was shaken at 250 rpm and 37 °C until OD_{600} reaches 1.5–3.0. The culture was cooled in an ice bath for 30 min. Protein expression was induced in the presence of 1 mM IPTG at 16 °C and shaken at 250 rpm for 16 to 24 h. The cells harvested by centrifugation were reconstituted in denaturing lysis buffer (100 mM NaH_2PO_4, 10 mM Tris, 9 M urea, 10 mM imidazole, pH 8.0) and then lysed by ultrasonication. The His_6-precursor in the supernatant was captured on HisPur Ni-NTA resin (Thermo Scientific, 625 mL per 20 mL supernatant) and purified according to the instructions provided by the manufacturer. The protein was eluted using NPI-250 (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH 8.0) and the buffer was exchanged into 50 mM Tris–HCl (pH 7.5) using a PD Minitrap G-10 column

(GE Healthcare). When XyeAB were expressed, the purified protein was digested by trypsin (10 µg per 1 mL eluate) at 37 °C for 16 h, or by GluC (10 µg per 1 mL eluate) at 25 °C for 16 h. Digested precursors were analyzed by LC-MS using the following conditions: column = Phenomenex Kinetex XB-C18, 5 µm, 150 x 4.6 mm; mobile phase/gradient = solvent A: H_2O (+0.1% formic acid, FA), solvent B: CH_3CN (+0.1% FA), isocratic 4% B for 2 min, followed by a linear gradient to 60% B over 10 min; flow rate = 0.5 mL/min; column temp. = 50 °C. When XyeAB and XyeCDE were coexpressed, the purified protein was directly analyzed by LC-MS using the following conditions: column = Phenomenex Aeris WIDEPORE C4, 3.6 µm, 150 x 4.6 mm; mobile phase/gradient = solvent A: H_2O (+0.1% formic acid, FA), solvent B: 1:1 CH_3CN/*i*-PrOH (+0.1% FA), isocratic 4% B for 2 min, followed by a linear gradient to 60% B over 12 min; flow rate = 0.5 mL/min; column temp. = 50 °C.

Purification of full-gene cluster expression by SPE and preparative HPLC

After the overnight protein expression by IPTG, cells were removed by centrifugation at 4,000 rpm for 15 min at 4 °C. 1 L supernatant was combined with 5.5 g of free-standing Strata-X® resin in a 2 L conical flask and shaken at 16 °C, 160 rpm to allow binding of the core peptide to the resin. Peptide-bound resin was then washed twice with 60% methanol (55 mL), 100% methanol (55 mL), and finally eluted with 60% CH3CN with 0.1% FA (55 mL). The elution fraction was concentrated in vacuo, reconstituted in 20% CH3CN with 0.1% FA, and subjected to purification by preparative HPLC at the following conditions: solvent A: H2O (+0.1% TFA), solvent B: CH3CN (+0.1% TFA) Kinetex XB-C18, 5 µm, 250 x 21.2 mm; isocratic 4% B for 1 min, followed by a linear gradient to 30% B over 22 min; flow rate = 20 mL/min; UV detection = 280 nm; column temp. = room temperature.

Purification of xenorceptides**.** After the overnight protein expression by IPTG, cells were removed by centrifugation at 4,000 rpm for 15 min at 4 °C. 1 L supernatant was combined with 5.5 g of free-standing Strata-X® resin in a 2 L conical flask and shaken at 16 °C, 160 rpm to allow binding of the core peptide to the resin. Peptide-bound resin was then washed twice with 60% methanol (55 mL), 100% methanol (55 mL), and finally eluted with 60% acetonitrile with 0.1% FA (55 mL). The elution fraction was concentrated in vacuo, reconstituted in 20% acetonitrile with 0.1% FA, and subjected to purification by preparative HPLC at the following conditions: column = Imtakt, Cadenza 5CD-C18, 5 µm, 250 x 20 mm; mobile phase/gradient = solvent A: H_2O

(+0.1% FA), solvent B: CH_3CN (+0.1% FA), isocratic 5% B for 1 min, followed by a linear gradient to 25% B over 17 min; flow rate = 21.2 mL/min; UV detection = 220 nm; column temp. = room temperature.

Yields of xenorceptides**.** Xenorceptide A1 (**1**) was obtained with yield of 5.0 mg/L of culture as a white powder. Xenorceptide A2 (**2**) was obtained with yield of 4.6 mg/L of culture as a white powder. Xenorceptide A3 (**3**) was obtained with yield of 1 mg/L of culture as a slightly yellow powder. Xenorceptide A4 (**4**) was obtained with yield of 3.3 mg/L of culture as slightly yellow powder.

Minimum inhibitory concentration (MIC) determination. MIC screening of the peptides against a panel of ATCC and clinical strains was performed using broth microdilution method.[1] Briefly, peptides stock solutions in DMSO (0.1% TFA) were diluted into Mueller Hinton Broth (MHB), followed by two-fold serial dilution in a 96-well plate. Bacteria culture in mid-log phase was diluted into MHB to yield 106 colony-forming units (CFU)/mL. Equal volume of the starting inoculum was added to the peptide samples, then incubated for 18–20 h (37 °C, 120 rpm). OD_{600} of the samples was then measured using Tecan Infinite M200 (TECAN, Männedorf, Switzerland). MIC is defined as the lowest peptide concentration to achieve more than 90% reduction in OD_{600} relative to the drug-free control. The experiments were repeated three times. Colistin-resistant clinical isolates are a kind gift from Dr. Jeanette Koh (National University Hospital, Singapore). Multidrug-resistant clinical isolates are a kind gift from Dr. Lakshminarayanan Rajamani (Singapore Eye Research Institute, Singapore).

Killing kinetics determination. Peptides stock solutions were diluted into MHB to desired concentrations. Bacteria culture in mid-log phase was diluted into MHB to yield 10^6 CFU/mL. The mixture was incubated at 37 °C with shaking. At each time point, 10 µL of the sample was drawn out and subjected to ten-fold serial dilution. 20 µL of relevant dilutions was dropped onto MHA plate using the drop plate method. The plate was incubated for 18–20 h at 37 °C. Colony number was counted, and used for calculating the CFU/mL according to the equation:
CFU/mL = Colony count × 50 × dilution factor

Field-emission scanning electron microscopy (FE-SEM) microscopy. *E. coli* M6 culture at mid-log phase was diluted to an OD_{600} of 0.1. After incubating the bacteria

with the peptide at 8×MIC for 1 h, 2 h, or 4 h at 37 °C with shaking, the samples were washed thrice in PBS. After overnight fixation with 2.5% glutaraldehyde (in PBS) at 4 °C, the samples were washed twice in PBS, and then re-suspended in 500 µL of PBS. Sample was dropped onto cover slips pre-treated with poly-l-lysine. After 30 min, unbound cells were washed away with PBS. Following post-fixation with 1% OsO_4 for 30 min, OsO_4 was removed, and the cover slips were washed twice with distilled water. Samples were dehydrated using a series of ethanol solutions (50%, 75%, 95%, 3 × 100%). They were then subjected to critical point drying using Leica EM CPD300 (Wetzlar, Germany), followed by sputter gold coating using Leica EM ACE200 (Wetzlar, Germany). Viewing of the samples was performed using JEOL JSM-6701F (Tokyo, Japan). Images were processed using ImageJ (National Institutes of Health, Bethesda, MD).

Serial passage. Resistance development of *E. coli* M6 against xenorceptide A2 was assessed by serial passaging of the bacteria in broth containing subinhibitory concentrations of the peptide. In brief, bacteria culture at mid-log phase was diluted to 10^5-10^6 CFU/mL in MHB containing 0.25×, 0.5×, 1×, 2×, and 4× MIC of the peptide. After 24h of incubation (37 °C, 120 rpm shaking), the new visually observed MIC value was recorded, and the culture at highest peptide concentration showing visible growth was diluted to 10^5-10^6 CFU/mL in MHB. A new set of peptide concentration range was added to the cultures based on the latest MIC. This process was repeated over 14 days for three independent starting cultures.

Advanced Marfey's analysis. 100 µg each of product was hydrolyzed in 6 M HCl (1 mL) at 110 °C for 18 h. The hydrolysate was concentrated using a centrifugal evaporator and reconstituted in water (100 µL), followed by addition of 1 M $NaHCO_3$ (40 µL) and 1% w/v of *N*α-(2,4-dinitro-5-fluorophenyl)-L-valinamide (L-FDVA) in acetone (200 µL). The mixture was incubated at 42 °C for 1 h and quenched with 2 M HCl (20 µL). L-Amino acid standards were derivatized in the same manner using L- and D-FDVA. The sample was diluted with CH_3CN/H_2O (1:1 v/v) and analyzed by LC-MS using negative ion mode. Retention times of the derivatized samples and standards are summarized in Table 15 with detailed LC conditions.

Table 15. Retention times of Marfey's type analysis of Xenorceptides.

	Retention time (min)[a]

Amino acid	L-DVA-std	D-DVA-std	Hydrolysate of **2**[b]	Hydrolysate of **3**[b]	Hydrolysate of **4**[b]
L-Ala	9.13	10.57	9.13	9.13	9.13
L-Arg	4.28	3.92	n.d.[c]	4.28	4.28
L-Asp	7.63	7.98	n.d.[c]	n.d.[c]	n.d.[c]
L-Ile	11.66	14.32	-	11.64	-
L-Lys	4.01	3.64	n.d.[c]	n.d.[c]	-
L-Phe	11.93	13.87	11.93	n.d.[c]	11.92
L-Ser	7.31	7.66	11.31	-	-
L-Thr	7.41	9.10	-	7.43	7.42
D-*allo*-Thr	7.66	8.44	-	-	-
L-Trp	11.53	12.77	n.d.[c]	n.d.[c]	n.d.[c]
L-Tyr	9.54	10.33	-	-	n.d.[c]
L-Val	10.60	13.04	n.d.[c]	-	n.d.[c]

[a]Analytical condition: MS polarity = negative; column: Kinetex XB-C18, 2.6 µm, 150 x 4.6 mm; flow rate: 0.50 mL/min; column temperature: 50 °C; mobile phase/gradient: 30% H_2O/CH_3CN + 0.1% FA isocratic for 2 min followed by linear gradient to 70% H_2O/CH_3CN + 0.1% FA over 17 min.

[b]Derivatized with L-FDVA.

[c]Not detected.

Derivatization of the hydrolysate of peptide 3 with GITC to resolve L-Ile and L-*allo*-Ile. 100 µg of hydrolysate of **3**, L-Ile, and L-*allo*-Ile were derivatized with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) using the same protocol as Marfey's type analysis described above except that GITC (200 µL, 1% in acetone) was used instead of L-FDVA and the reaction was placed at room temperature for 1 h. The samples were then diluted with 1:1 ACN/H_2O and analyzed by LCMS using negative mode. The retention times are given in Table 16 with detailed LC condition.

Table 16. Retention times of GITC derivatization of **3**.

	Retention time (min)[a]		
Amino acid	L-std[b]	L-*allo*-std[b]	Hydrolysate of **3**[b]
Ile	10.32	10.26	10.31

[a]Analytical condition: MS polarity = negative; column: Kinetex XB-C18, 2.6 µm, 150 x 4.6 mm; flow rate: 0.50 mL/min; column temperature: 50 °C; mobile phase/gradient: 30% H_2O/CH_3CN + 0.1% FA isocratic for 2 min followed by linear gradient to 70% H_2O/CH_3CN + 0.1% FA over 17 min.

[b]Derivatized with GITC.

Table 17. High-resolution MS data of modified peptide products identified in this study.

SEQ ID	Compound #	Sequence[a]	Charge State	Calculated mass (monoisotopic)	Observed mass (monoisotopic)	Δppm
32	**1**	WINAFGNWERAFH	$[M+2H]^{2+}$	821.3709	821.3721	1.5
8	**2**	WVNAFARWSKSF	$[M+2H]^{2+}$	746.8597	746.8602	0.7
13	**3**	WINAFANWTKRI	$[M+2H]^{2+}$	757.3886	757.3889	0.4
25	**4**	WVNAYARWTNRF	$[M+2H]^{2+}$	789.3735	789.3741	0.8
225	**S1**	ELVDSLLDTVSGGWINAFGNWERAFH	$[M+3H]^{3+}$	976.4631	976.4649	1.8
226	**S2**	ALAQSMLDSVSGGWVNAFARWSKSF	$[M+3H]^{3+}$	903.7675	903.7661	-1.5
227	**S3**	ILVDSLLDTVSGGWINAFANWTKRI	$[M+3H]^{3+}$	928.4887	928.4896	1.0
228	**S4**	NNQPQPLTEDLLDQISGGWVNAYARWTNRF	$[M+3H]^{3+}$	1166.5589	1166.5593	0.3

[a]Cyclized three-residue motifs are indicated in red.

In vivo efficacy in peritonitis model.

All animal procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) at National University of Singapore (Singapore). Female C57BL/6NTac mice aged 6-8 weeks were acquired from InVivos Pte Ltd (Singapore, Singapore). Solutions for injections were prepared fresh in pharmaceutical grade saline and filter-sterilized. Murine peritonitis model was established according to literature. Briefly, healthy mice were rendered neutropenic by administering i.p. injection (0.5 mL) of cyclophosphamide on day -4 (150 mg/kg) and day -1 (100 mg/kg). On day 0, mice were infected with E. coli M6 (109 CFU/mL) through i.p. injection (0.1 mL). At 30 min post-inoculation, mice were given i.p. injection (0.5

mL) of a single dose of Smc (5 or 50 mg/kg), colistin (5 mg/kg), or saline control (n = 5 mice per treatment group). At 2 h post-treatment, mice were humanely euthanized by carbon dioxide asphyxiation and cervical dislocation. Sterile PBS (3 mL) was injected into the peritoneal cavity, followed by abdominal massage and collection of peritoneal fluid (1-2 mL). Blood (0.3-0.5 mL) was collected through cardiac puncture. Liver, spleen, and kidney were surgically removed and stored in 0.1% Triton X-100 (in PBS). Tissue homogenization was performed using gentleMACS dissociator (Miltenyi Biotec, Germany) by following a published protocol. Cell aggregates were removed using a 30 µm mesh MACS SmartStrainer (Miltenyi Biotec). Blood, peritoneal fluid, and tissue homogenates were plated on LB agar and incubated overnight for colony counting.

LC-MS experiments

Mobile phases used are as follows: (A1) H2O + 0.1% formic acid; (B1) CH3CN + 0.1% formic acid; (B2) 1:1 CH3CN/isopropanol + 0.1% formic acid. Details of conditions used for various samples are listed below:

For full-length precursors analyses, 10 µL of sample was injected into the system and left to run with the Phenomenex® Aeris Widepore 3.6 µm C4 column (150 x 4.6 mm) as stationary phase and mobile phases of A1 and B2 were used at a flow rate of 0.5 mL/min for 20 minutes and 10-75% B2 gradient over 12.5 minutes.

For digested fragment analyses, 40 µL of sample was injected into the system and left to run with Phenomenex Kinetex XB-C18, 5 µm, 150 x 4.6 mm column (150 x 4.6 mm) as stationary phase and mobile phases of A1 and B1 were used at a flow rate of 0.5 mL/min for 25 minutes and 4-60% B1 gradient over 17 minutes.

For SPE fractions, 40 µL of sample was injected into the system and left to run with Phenomenex Kinetex XB-C18, 5 µm, 150 x 4.6 mm column (150 x 4.6 mm) as stationary phase and mobile phases of A1 and B1 were used at a flow rate of 0.5 mL/min for 15 minutes and 4-32% B1 gradient over 7 minutes.

For subsequent MS/MS of fragmentation of selected ions, a collision energy of 30-45 eV was used. MassLynx v.4.1 was finally used to analyze the data collected.

Antimicrobial Assays

MIC values for compounds (1–11) were assessed using 96–well plate format with Mueller Hinton (MH) broth, using the two-fold dilution method, previously reported in standard methods provided by Clinical and Laboratory Standards S8 Institute (CLSI). Kanamycin and ampicillin were used as antibacterial control agents. According to the

reference, the compounds (1–11) were first dissolved in DMSO + 0.1%TFA at a concentration of 3.2 mg/mL and 4 µL was serially diluted in 96 µL of MH broth. Then, sequential 2-fold serial dilutions of the mix were diluted in 50 µL MH broth and 50 µL cell cultures were added to wells. After incubation at 37 °C for 18 h, the lowest concentrations that completely inhibited the growth of bacteria in microdilution wells were detected by microplate reader for each tested compound, the values were recorded in Table 14. All assays were carried out in triplicate.

General cyclophane synthetic protocol

Precursor peptide containing alkyne moiety and 2-bromoacetanilide moiety (1.00 g, 1.04 mmol, 1.0 equiv) and $Pd(PtBu_3)_2$ (180 mg, 0.347 mmol, 0.3 equiv) were added to a flame-dried round bottom flask. The flask was evacuated and backfilled with argon (3X). Dry dioxane (100 mL) and DIPEA (0.99 mL, 5.20 mmol, 5.0 equiv) were added and the mixture was heated to 85 °C. After 1.5 h, the reaction solution was cooled to ambient temperature then evaporated under vacuum. The crude solid may be purified via flash column chromatography using a gradient of 30% to 90% EtOAc in DCM.

Table 18. NMR data for **xenorceptide A2**.

Residue	Position	$^{1}H^{a}$	$^{13}C^{a,b}$	COSY	HMBC (H to C)	NOESY
Trp1	C=O		168.3			
	NH_2	8.22		Hα		Trp1-Hα
	α	3.65	54.5	NH_2, Hβ		Trp1-NH_2, Trp1-Hβa, Tryp1-Hβb, Val2-NH
	β	3.10 (Ha)	27.0	Hα	Trp1-Ca, Trp1-C2, Trp1-C3, Trp1-C3a	Trp1-Hα, Trp1-H4
		3.06 (Hb)				Trp1-Hα, Trp1-H2
	1	10.80		H2	Trp1-C2, Trp1-C3, Trp1-C3a, Trp1-C7a	Trp1-H2, Trp1-H7
	2	7.18	124.6	H1	Trp1-C3a, Trp1-C7a	Trp1-H1, Tryp1-Hβb
	3		108.0			
	3a		127.2			
	4	7.13	116.4	H5	Trp1-C3, Trp1-C3a, Trp1-C6, Trp1-C7a	Trp1-Hβa, Trp1-H5
	5	6.77	124.2	H4, H7	Trp1-C3a, Trp1-C7	Trp1-H4, Asn3-NH, Asn3-Hβ
	6		130.9			

	7	7.38	110.7	H5	Trp1-C3a, Trp1-C5, Asn3-Cb	Trp1-H1
	7a		137.1			
Val2	C=O		168.5			
	NH	6.94		Hα	Trp1-C=O	Trp1-Hα, Val2-Hβ
	α	3.77	57.0	NH, Hβ	Val2-C=O, Val2-Cβ, Val2-Cγ-M1	Val2-Hβ, Val2-Hγ-M1, Asn3-NH
	β	1.45	31.9	Hα, Hγ, Hγ–M1, Hγ–M2	Val2-C=O, Val2-Cα, Val2-Cγ-M1	Val2-Hγ-M1, Val2-Hγ–M2
	γ-M1	0.70	18.4	Hβ	Val2-Cα, Val2-Cβ	Val2-Hβ
	γ-M2	0.68	18.4	Hβ	Val2-Cα, Val2-Cβ	Val2-Hβ
Asn3	C=O		169.6			
	NH	7.67		Hα	Val2-C=O	Trp-H5, Val2-Hα
	α	4.71	55.9	NH, Hβ	Val2-C=O, Asn3-Cβ, Asn3-$CONH_2$, Asn3-C=O	Ala4-NH
	β	3.74	52.0	Hα	Trp1-C5, Trp1-C6, Trp1-C7, Asn3-$CONH_2$, Asn3-Cα, Asn3-C=O	Trp1-H5
	$CONH_2$		173.8			
Ala4	C=O		171.7			
	NH	7.24		Hα	Asn3-C=O	Asn3-Hα, Ala4-Hα, Ala4-Hβ
	α	4.40	48.1	NH, Hβ	Ala4-Cβ	Ala4-NH, Ala4-Hβ, Phe5-NH
	β	1.13	18.4	Hα, Hγ	Ala4-Cα, Ala4-C=O	Ala4-NH, Ala4-Hα Phe5-NH
Phe5	C=O		n.d.[c]			
	NH	8.08		Hα		Ala4-Hα, Ala4-Hβ, Phe5-Hα, Phe5-Hβ
	α	4.26	54.5	NH, Hβ		Phe5-Hα, Phe5-Hβ, Phe5-H6, Ala6-NH

	β	2.96 (Ha)	39.5	Hα		Phe5-NH, Phe5-H2, Phe5-H6
		2.73 (Hb)				Phe5-NH, Phe5-H2
	1		n.d.[c]			
	2	6.91	133.3	H5	Phe5-Cβ, Phe2-C6, Arg7-Cβ	Phe5-Hβa, Phe5-Hβb, Arg7-NH, Arg7-Hβ
	3		n.d.[c]			
	4	7.17	123.4	H6	Phe2-C2, Phe2-C6	Arg7-Hγ
	5	7.25	129.1	H2		Phe5-H4, Phe5-H6
	6	7.09	127.6	H3		Phe5-H5, Phe5-Hα, Phe5-Hβa
Ala6	C=O		169.9			
	NH	7.86		Hα		Phe5-Hα
	α	4.38	46.4	NH, Hβ	Ala6-Cβ	Ala6-Hβ, Arg7-NH
	β	0.95	15.8	Hα	Ala6-Cα, Ala6-C=O	Ala6-Hα
Arg7	C=O		n.d.[c]			
	NH	7.58		Hα		Phe5-H2, Ala6-Hα
	α	4.23	58.3	NH, Hβ		Arg7-Hβ, Arg7-Hγ, Trp8-NH
	β	2.87	45.7	Hα	Arg7-Cδ	Phe5-H2, Arg7-Hα, Trp8-NH
	γ	2.10 (Ha)	28.3			Phe5-H4, Arg7-Hα
		1.94 (Hb)				Phe5-H4, Arg7-Hα
	δ	2.96	37.2			
	C (guanidine)		n.d.[c]			
Trp8	C=O		170.6			
	NH	8.53		Hα		Arg7-Hα, Arg7-Hβ, Trp8-Hβ
	α	3.89	57.0	NH, Hβ		Trp8-Hβ, Thr9-NH
	β	3.02 (Ha)	28.3	Hα	Trp8-C3	

		2.98 (Hb)				Trp8-NH, Trp8-Hα
	1	10.70		H2	Trp8-C2, Trp8-C3, Trp8-C3a, Trp8-C7a	Trp8-H2, Trp8-H7
	2	7.16	123.9	H1	Trp8-C7a	Trp8-NH
	3		110.3			
	3a		128.2			
	4	7.14	115.9	H5	Trp8-C6, Trp8-C7α	Trp8-H5
	5	6.77	124.6	H4	Trp8-C3a, Trp8-C7	Trp8-H4, Lys10-NH, Lys10-Hβ
	6		132.9			
	7	7.17	110.4		Arg10-Cβ	Trp8-H1, Lys10-Hα
	7a		137.8			
Ser9	C=O		167.9			
	NH	5.84		Hα		Trp8-Hβ
	α	4.03	54.5	NH, Hβ	Trp8-C=O, Ser9-Cβ, Ser9-C=O	Ser9-Hβ, Lys10-NH
	β	3.09	62.0	Hα	Ser9-C=O	Ser9-NH, Lys10-NH
Lys10	C=O		170.7			
	NH	7.42		Hα		Trp8-H5, Ser9-Hα, Lys10-Hα, Lys10-Hβ
	α	4.16	60.7	NH, Hβ	Trp8-C6, Ser9-C=O, Lys10-C=O, Lys10-Cβ, Lys10-Cγ	Trp8-H7, Lys10-NH, Lys10-Hγa, Lys10-Hγb, Ser11-NH
	β	2.73	49.5	Hα, Hγ		Trp8-H5, Lys10-Hα, Lys10-Hγa, Lys10-Hgb, Lys10-Hδa, Lys10-Hδb
	γ	1.97 (Ha)	24.5	Hβ, Hδ		Lys10-Hα, Lys10-Hβ
		1.86 (Hb)				Lys10-Hα, Lys10-Hβ
	δ	1.74 (Ha)	25.7	Hγ, Hε		Lys10-Hβ
		1.50 (Hb)				Lys10-Hβ
	ε	2.75	39.4	NH_2, Hδ		Lys10-NH_2
	NH_2	7.64		Hε		Lys10-Hε

Ser11	C=O		n.d.[c]			
	NH	8.31		Hα		Lys10-Cα, Ser11-Hβ
	α	4.32	55.7	NH, Hβ		Ser11-Hβ, Phe12-NH
	β	3.58	61.9	Hα, Hγ		Ser11-NH
Phe12	C=O		173.2			
	NH	8.15		Hα		Ser11-Hα, Phe12-Hβb
	α	4.42	53.3	NH, Hβ		Phe12-NH
	β	3.05	36.9		Phe12-Cα, Phe12-C1, Phe12-C2, Phe12-C=O	
		2.96				Phe12-NH
	1		137.3	Hα, Hγ		
	2	7.26	129.2	Hβ, Hδ	Phe12-Cβ, Phe12-C4, Phe12-C6	
	3	7.29	128.8	Hβ	Phe12-C1, Phe12-C5	
	4	7.24	127.0	Hγ	Phe12-C2, Phe12-C6	
	5	7.29	128.7		Phe12-C1, Phe12-C5	
	6	7.26	129.2		Phe12-Cβ, Phe12-C4, Phe12-C6	

[a]800 MHz in DMSO-d_6 at 298 K. [b]Assigned by HSQC and HMBC. [c]Not detected.

Table 19. NMR data for **xenorceptide A3**.

Residue	Position	$^{1}H^{a}$	$^{13}C^{a,b}$	COSY	HMBC (H to C)	NOESY
Trp1	C=O		167.7			
	NH_2	8.26		Hα		Trp1-Hβ
	α	3.65	54.8	NH_2, Hβ		Ile2-NH
	β	3.08	27.4	Hα	Trp1-C3, Trp1-C3a, Trp1-C=O	Trp1-NH_2, Trp1-Hα, Trp1-H2
	1	10.80		H2	Trp1-C2, Trp1-C3, Trp1-C3a, Trp1-C7a	Trp1-H2, Trp1-H7
	2	7.16	123.9	H1	Trp1-C3, Trp1-C3a, Trp1-C7a	Trp1-Hβ, Trp1-H1
	3		107.5			

	3a		126.8			
	4	7.13	116.0	H5	Trp1-C6, Trp1-C7a	Trp1-H5
	5	6.78	123.9	H4, H7	Trp1-C3a, Trp1-C7, Asn3-Cβ	Trp1-H4, Asn3-Hβ
	6		130.3			
	7	7.39	110.8	H5	Trp1-C3a, Trp1-C5, Asn3-Cβ	Trp1-H1, Asn3-Hα
	7a		136.5			
Ile2	C=O		167.8			
	NH	6.92		Hα	Trp1-C=O	Trp1-Hα
	α	3.80	56.7	NH, Hβ	Ile2-Cβ, Ile2-Cγ-ε	Asn3-NH,
	β	1.19	38.5	Hα, Hγ		Ile2-Hγ–Mε
	γ	1.32	24.1	Hβ, Hδ		Ile2-Hδ
	γ–Mε	0.66	14.8	Hβ	Ile2-Cα, Ile2-Cb, Ile2-Cγ	Ile2-Hα, Ile2-Hβ
	δ	0.72	11.0	Hγ	Ile2-Cβ, Ile2-Cγ	Ile2-Hγ
Asn3	C=O		169.2			
	NH	7.65		Hα		Ile2-Hα
	α	4.72	56.4	NH, Hβ	Ile2-CO, Asn3-Cβ, Asn3-$CONH_2$, Asn3-C=O	Trp1-H7, Ala4-NH,
	β	3.77	52.5	Hα	Trp1-C5, Trp1-C6, Trp1-C7, Asn3-$CONH_2$, Asn3-Cα	Trp1-H5
	$CONH_2$		173.1			
Ala4	C=O		171.1			
	NH	7.40		Hα	Asn3-C=O	Asn3-Hα
	α	4.37	47.7	NH, Hβ	Ala4-Cβ, Ala4-C=O	Ala4-Hβ, Phe5-NH
	β	1.13	18.6	Hα, Hγ	Ala4-Cα, Ala4-C=O	Ala4-Hα
Phe5	C=O		n.d.[c]			
	NH	7.98		Hα	Ala4-C=O	Ala4-Hα
	α	4.50	54.6	NH, Hβ		Ala6-NH,
	β	3.20 (Ha)	38.6	Hα		Phe5-Hβb, Phe5-H6
		2.56 (Hb)				Phe5-Hβa, Phe5-H6

	1		135.6			
	2	6.85	129.2	H3	Phe5-C4, Phe5-C6	Phe5-Hβa, Phe5-Hβb, Phe5-H3
	3	7.03	131.5	H2	Phe5-C1, Phe5-C3, Asn7-Cβ	Phe5-H2, Asn7-Hβ
	4		136.2			
	5	7.19	126.2		Phe5-C1, Phe5-C3	
	6	7.16	129.0			
Ala6	C=O		171.2			
	NH	6.88		Hα		Phe5-Hα
	α	3.72	48.2	NH, Hβ		Asn7-NH
	β	0.96	19.0	Hα	Ala6-Cα, Ala6-C=O	
Asn7	C=O		172.4			
	NH	7.81		Hα		Ala6-Hα, Asn7-Hβ
	α	5.05	53.8	NH, Hβ	Ala6-C=O, Asn7-Cβ, Asn7-$CONH_2$, Asn7-C=O	Trp8-NH
	β	3.75	52.5	Hα	Phe5-C3, Phe5-C4, Phe5-C5, Asn7-$CONH_2$, Asn7-C=O	Phe5-H5, Asn7-NH
	$CONH_2$					
Trp8	C=O		n.d.[c]			
	NH	7.12		Hα		Asn7-Hα, Trp8-Hα
	α	3.94	56.9	NH, Hβ		Trp8-NH, Thr9-NH
	β	3.00 (Ha)	29.1	Hα		Trp8-H2
		2.88 (Hb)				Trp8-H2
	1	10.69		H2	Trp8-C3, Trp8-C3a, Trp8-C7a	
	2	7.12	123.1	H1	Trp8-C3, Trp8-C4, Trp8-C7a	Trp8-Hβa, Trp8-Hβb
	3		109.3			
	3a		127.5			
	4	7.10	116.3	H5	Trp8-C7a, Trp8-C6	Trp8-H5

	5	6.70	124.7	H4	Trp8-C3a, Trp8-C7, Lys10-Cβ	Trp8-H4, Lys10-Hβ
	6		132.3			
	7	7.16	109.8		Trp8-C5, Lys10-Cβ	Lys10-Hα, Lys10-Hγa, Lys10-Hγb
	7a		137.1			
Thr9	C=O		166.8			
	NH	5.95		Hα		Trp8-Hα
	α	3.93	57.6	NH, Hβ	Thr9-C=O	Thr9-Hβ, Thr9-Hγ, Lys10-NH
	β	3.35	67.5	Hα	Thr9-C=O	Thr9-Hα, Thr9-Hγ
	γ	0.72	19.2		Thr9-Cα, Thr9-Cβ	Thr9-Hα, Thr9-Hβ
Lys10	C=O		170.2			
	NH	7.30		Hα		Thr9-Hα
	α	4.12	60.0	NH, Hβ	Lys10-C=O	Trp8-H7, Lys10-Hγ, Arg11-NH
	β	2.68	49.2	Hα, Hγ		Trp8-H5
	γ	1.98 (Ha)	24.9	Hβ, Hδ		Lys10-Hγb, Trp8-H7, Lys10-Hα
		1.78 (Hb)				Lys10-Hγa, Trp8-H7, Lys10-Hα
	δ	1.53	26.2	Hγ, Hε	Lys10-Cε	
	ε	2.78	38.7	NH_2, Hδ		Lys10-NH_2
	NH_2	7.74		Hε		Lys10-Hε
Arg11	C=O		171.4			
	NH	8.38		Hα	Lys10-C=O	Lys10-Hα, Arg11-Hα, Arg11-Hβ
	α	4.32	52.3	NH, Hβ		Arg11-NH, Arg11-Hβ, Arg11-Hγ, Ile12-NH,
	β	1.66 (Ha)	28.8	Hα, Hγ		Arg11-NH
		1.52 (Hb)				
	γ	1.50	25.6	Hβ, Hd		Arg11-Hα, Arg11-Hδ
	δ	3.09	40.4	Hγ	Arg11-C (guanidine)	Arg11-Hγ

	C (guanidine)		156.8			
Ile12	C=O		172.8			
	NH	8.06		Hα	Arg11-C=O	Arg11-Ha
	α	4.23	56.2	NH, Hβ	Arg11-C=O, Ile12-Cβ, Ile12-Cγ, Ile12-Cγ–Mε, Ile12-C=O	Ile12-NH, Ile12-Hβ
	β	1.83	36.4	Hα, Hγ		Ile12-Ha, Ile12-Hδ, Ile12-Hγ–Mε
	γ	1.23	24.3	Hβ, Hδ	Ile12-Cβ, Ile12-Cγ–Mε, Ile12-Cδ	
	γ–Mε	0.89	15.5	Hβ	Ile12-Cα, Ile12-Cβ, Ile12-Cγ	Ile12-Hβ
	δ	0.86	11.1	Hγ	Ile12-Cβ, Ile12-Cγ	Ile12-Hβ

[a]400 MHz in DMSO-d_6 + 0.3% TFA-*d* at 298 K. [b]Assigned by HSQC and HMBC. [c]Not detected.

Table 20. NMR data for **xenorceptide A4**.

Residue	Position	$^{1}H^{a}$	$^{13}C^{a,b}$	COSY	HMBC (H to C)	NOESY
Trp1	C=O		167.7			
	NH_2	8.24		Hα		Trp1-Hα, Trp1-Hβ
	α	3.65	54.6	NH_2, Hβ		Trp1-NH_2, Val2-NH
	β	3.09	27.3	Hα		Trp1-NH_2, Trp1-H4
	1	10.80		H2	Trp1-C3, Trp1-C3a, Trp1-C7a	Trp1-H2, Trp1-H7
	2	7.17	123.6	H1	Trp1-C3, Trp1-C3a	Trp1-H1
	3		107.3			
	3a		126.5			
	4	7.13	115.8	H5	Trp1-C6, Trp1-C7a	Trp1-Hb, Trp1-H5
	5	6.77	123.7	H4	Trp1-C3a, Trp1-C7, Asn3-Cβ	Trp1-H4, Asn3-Hβ, Asn3-NH
	6		130.1			
	7	7.38	110.6		Trp1-C3a, Trp1-C5, Asn3-Cβ	Trp1-H1, Asn3-Hα
	7a		136.6			
Val2	C=O		167.8			
	NH	6.95		Hα	Trp1-C=O	Trp1-Hα

	α	3.77	57.3	NH, Hβ	Val2-C=O	Asn3-NH
	β	1.45	32.0	Hα, Hγ–M1, Hγ–M2	Val2-Cγ–M1, Val2-Cγ–M2	Val2-Hγ-M1, Val2-Hγ–M2
	γ-M1	0.69	18.9	Hβ, Hδ	Val2-Cα, Val2-Cβ, Val2-Cγ–M2	Val2-Hβ
	γ-M2	0.68	18.4	Hβ	Val2-Cα, Val2-Cβ, Val2-Cγ–M1	Val2-Hβ
Asn3	C=O		168.5			
	NH	7.65		Hα	Val2-Cα	Val2-Hα, Trp1-H5
	α	4.73	56.1	NH, Hβ	Asn3-C=O	Trp1-H7, Ala4-NH
	β	3.74	52.4	Hα	Trp1-C5, Trp1-C6, Trp1-C7, Asn3-Cα	Trp1-H5
	$CONH_2$					
Ala4	C=O		170.8			
	NH	7.27		Hα		Asn3-Hα
	α	4.39	47.4	NH, Hβ		Ala4-Hβ, Tyr5-NH
	β	1.13	18.6	Hα, Hγ	Ala4-Cα, Ala4-C=O	Ala4-Hα, Tyr5-NH
Tyr5	C=O		n.d.[d]			
	NH	8.04		Hα		Ala4-Hα, Ala4-Hβ, Tyr5-Hβa, Tyr5-Hβb
	α	4.16	55.3	NH, Hβ		Ala6-NH
	β	2.84 (Ha)	38.1	Hα		Tyr5-NH, Tyr5-Hβb, Tyr5-H2, Tyr5-H6
		2.62 (Hb)				Tyr5-NH,Tyr5-Hβa, Tyr5-H2, Tyr5-H6
	1		125.6[c]			
	2	6.67	135.3			Tyr5-Hβa, Tyr5-Hβb, Arg3-Hβ
	3		123.6[c]			
	4		154.9			
	5	6.66	115.8	H6	Tyr5-C1, Tyr5-C3	Tyr5-H6, Tyr5-OH
	6	6.89	128.2	H5	Tyr5-C2, Tyr5-C4	Tyr5-Hba, Tyr5-Hβb, Tyr5-H5

	OH	9.39				Tyr5-H5
Ala6	C=O		n.d.[d]			
	NH	7.68		Hα		Tyr5-Hα, Ala6-Hβ
	α	4.34	46.3	NH, Hβ		Ala6-Hβ, Asn7-NH
	β	0.93	15.9	Hα		Ala6-NH
Arg7	C=O		n.d.[d]			
	NH	7.39		Hα		Ala6-Hα, Trp8-NH
	α	4.54	54.7	NH, Hβ		Trp8-NH
	β	2.69	46.2	Hα		Arg7-Hγ
	γ	2.54 (Ha) 1.75 (Hb)	27.3			Arg7-Hβ, Arg7-Hδ
	δ	2.91	39.7			Arg7-Hγ
	C (guanidine)		n.d.			
Trp8	C=O		n.d.[d]			
	NH	8.64		Hα		Arg7-NH, Arg7-Hα, Trp8-Hβ
	α	3.85	57.7	NH, Hβ		Trp8-Hβ, Thr9-NH
	β	3.01	28.1	Hα		Trp8-NH, Trp8-Hα, Trp8-H2, Trp8-H4
	1	10.72		H2	Trp8-C3, Trp8-C3a	Trp8-H2, Trp8-H7
	2	7.15	123.3	H1	Trp8-C3, Trp8-C7a	Trp8-NH
	3		109.7			
	3a		126.9			
	4	7.18	116.2	H5	Trp8-C6	Trp8-Hβ, Trp8-H5
	5	6.73	123.5	H4	Trp8-C3a	Trp8-H4, Lys10-NH, Lys10-Hβ
	6		130.0			
	7	7.32	110.8		Trp8-C3a, Trp8-C5, Asn10-Cβ	Trp8-NH, Lys10-Hα
	7a		136.4			

Thr9	C=O		167.2			
	NH	6.06		Hα		Trp8-Hα
	α	3.90	57.5	NH, Hβ		Asn10-NH
	β	3.41	67.5	Hα, Hγ		Thr9-Hγ, Asn10-NH
	γ	0.81	18.7	Hβ	Thr9-Cα, Thr9-Cβ	Thr9-Hβ
Asn10	C=O		169.5			
	NH	7.55		Hα		Trp8-H5, Thr9-Hα, Thr9-Hβ
	α	4.77	56.0	NH, Hβ	Asn10-C=O	Trp8-H7, Arg11-NH
	β	3.73	52.5	Hα, Hγ		Trp8-H5
	$CONH_2$		n.d.[d]			
Arg11	C=O		170.8			
	NH	7.48		Hα	Asn10-C=O	Asn10-Cα, Arg11-Hα, Arg11-Hβ
	α	4.29	51.4	NH, Hβ		Arg11-NH, Arg11-Hβ, Phe12-NH
	β	1.63 (Ha) 1.42 (Hb)	29.0	Hα, Hγ		Arg11-NH, Arg11-Hα, Phe12-NH
	γ	1.40	24.3	Hβ, Hδ		Arg11-Hδ
	δ	3.01	40.3	Hγ		Arg11-Hγ
	C (guanidine)		n.d.[d]			
Phe12	C=O		172.4			
	NH	8.16		Hα	Arg11-C=O	Arg11-Hα, Arg11-Hβ, Phe12-Hα, Phe12-Hβ
	α	4.38	53.4	NH, Hβ	Phe12-Cβ, Phe12-C1, Phe12-C=O	Phe12-NH
	β	3.06 3.00	36.4	Hα	Phe12-C=O	Phe12-NH
	1	137.2				

	2	128.9	7.27		Phe12-Cβ, Phe12-C4, Phe12-C6	
	3	128.1	7.29	H4	Phe12-C1, Phe12-C5	
	4	126.2	7.21	H3, H5	Phe12-C2, Phe12-C6	
	5	128.1	7.29	H4	Phe12-C1, Phe12-C5	
	6	128.9	7.27		Phe12-Cβ, Phe12-C4, Phe12-C6	

[a]400 MHz in DMSO-d_6 + 0.2% TFA-*d* at 298 K. [b]Assigned by HSQC and HMBC. [c]The assignment of Tyr5-C1 and Tyr5-C3 are interchangeable. [d]Not detected.

Table 21 NMR data for xenorceptide D1.

Residue	Position	$^{1}H^{a}$	$^{13}C^{b}$	COSY	HMBC (H to C)	NOESY
Arg(-4)	C=O		18.9			
	NH	8.22		Hα	Arg(-4)-CO	
	α	3.86	42.2	NH, Hβ		
	β	3.20	40.2	Hα, Hγ		
	γ	1.53 (Ha) 1.72 (Hb)	26.6	Hβ, Hδ		
	δ	2.70	39.2	Hγ		
Gly(-3)	C=O		168.8			
	NH	8.71		Hα		
	α	3.88	42.18	NH, Hβ		
Glu(-2)	C=O		172.1			
	NH	8.20		Hα		
	α	4.30	52.5	NH, Hβ		
	β	1.78 (Ha) 1.93 (Hb)	28.0	Hα, Hγ, OH		
	γ	2.28 (Ha) 2.30 (Hb)	30.5	Hβ		
Gly(-1)	C=O		168.2			
	NH	8.20		Hα	Gly(-1)-CO	
	α	3.86	42.2	NH, Hβ		Trp1-NH
Trp1	C=O		168.2			
	NH	7.98		Hα	Gly(-1)-CO	Gly(-1)-Hα, Trp1-Hα, Trp1-Hβ

	α	3.94	57.4	Hβ, NH		Val2-NH, Trp1-Hβ, Trp1-H4
	β	2.94	29.4	Hα	Trp1-C3a	Val2-NH, Trp1-Hα, Trp1-H2, Trp1-H4
	4	7.15	116.7	H5	Trp1-C3, Trp1-C3a, Trp1-C5, Trp1-C6, Trp1-C7a	Trp1-Hβ, Trp1-H5
	5	6.72	125.1	H4	Arg3-Cβ, Trp1-C3a, Trp1-C7	Arg3-Hβ, Trp1-H7
	6		132.4			
	7	7.14	110.0		Arg3-Cβ, Trp1-C3, Trp1-C3a, Trp1-C5, Trp1-C6, Trp1-C7	Arg3-Hβ, Trp1-H5
	7a	137.5				
	1	10.74		H2	Trp1-C2, Trp1-C7, Trp1-C7a	Trp1-H2
	2	7.16	123.7	NH	Trp1-C3, Trp1-C3a, Trp1-C7a	Trp1-Hβ, Trp1-NH
	3		110.1			
	3a		128.2			
Val2	C=O		171.7			
	NH	5.96		Hα		Trp1-Hα, Val2-Hγ1, Val2-Hγ2
	α	3.77	57.2	NH, Hβ	Val2-CO, Arg3-CO, Val2-Cβ	Val2-Hβ, Val2-Hγ1, Val2-Hγ2, Arg3-Hα
	β	1.36	32.5	Hα, Hγ1, Hγ2	Val2-Cα, Val2-Cγ1, Val2-Cγ2,	Val2-NH, Val2-Hα, Val2-Hγ1, Val2-Hγ2, Arg3-NH
	γ1	0.54	19.3	Hβ	Val2-Cα, Val2-Cβ, Val2-Cγ2	Val2-Hα, Val2-Hβ
	γ2	0.60	18.6	Hβ	Val2-Cα, Val2-Cβ, Val2-Cγ1	Val2-Hα, Val2-Hβ
Arg3	C=O		170.5			
	NH	7.49		Hα		Val2-Hα, Val2-Hβ, Arg3-Hβ
	α	4.08	60.5	NH,Hβ		Ala4-NH
	β	2.82	46.4	Hα, Hγ		Ala4-NH

	γ	2.13	28.0	Hβ, Hδ		Arg3-Hα, Arg3-Hβ, Arg3-Hδ,
	δ	3.20	40.3	NH		Arg3-Hγ
	NH (side chain)	7.45		Hδ		Arg3-Hδ
Ala4	C=O		172.3			
	NH	8.20		Hα	Ala4-CO	Ala4-Hα, Ala4-Hβ
	α	4.22	48.7	NH, Hβ	Ala4-Cβ, Ala4-CO	Ala4-Hβ, Tyr5-NH
	β	1.20	18.9	Hα	Ala4-Cα, Ala4-CO	Ala4-Hα, Ala4-NH
Tyr5	C=O		173.0			
	NH	7.75		Hα		Tyr5-Hα, Tyr5-Hβ
	α	4.57	51.6	NH, Hβ	Tyr5-CO	
	β	2.62 (Ha) 2.12 (Hb)	35.0	Hα	Tyr5-Cα, Tyr5-C1	Tyr5-NH, Tyr5-H2, Tyr5-H6
	1		131.1			
	2	7.04	130.9	H3	Tyr5-Cβ, Tyr5-C1, Tyr5-C3, Tyr5-C5, Tyr5-C4, Tyr5-C6	Tyr5-Hα, Tyr5-Hβ, Tyr5-H3
	3	6.63	115.37	H2	Tyr5-C2, Tyr5-C5, Tyr5-C6	Tyr5-H2
	4		156.5			
	5	6.63	115.37	H6	Tyr5-C2, Tyr5-C3, Tyr5-C6	Tyr5-H6
	6	7.04	130.9	H5	Tyr5-Cβ, Tyr5-C1, Tyr5-C2, Tyr5-C3, Tyr5-C4, Tyr5-C5	Tyr5-Hα, Tyr5-Hβ, Tyr5-H5
	OH	9.21			Tyr5-C3, Tyr5-C4, Tyr5-C5	Tyr5-H3, Tyr5-H5
Trp6	C=O		169.0			
	NH	8.72		Hα	Trp6-CO	
	α	3.88	42.1	NH, Hβ (Ha), Hβ (Hb),	Trp6-CO	Ala7-NH
	β	2.92 (Ha) 2.89 (Hb)	29.4	Hα	Trp6-Cα, Trp6-C3a	Trp6-H2
	4	7.11	116.9	H5	Trp6-C3a, Trp6-C3a, Trp6-C6, Trp6-C7, Trp6-C7a	Trp6-Hβ(Hb)

	5	6.75	125.1	H4	Lys8-Cβ, Trp6-C3a, Trp6-C7	Trp6-H4, Lys8-Hα, Lys8-Hβ
	6		132.6			
	7	7.15	110.2		Lys8-Cβ, Trp6-C3a, Trp6-C5, Lys8-C6, Trp6-C7a	Trp6-H5, Lys8-Hα, Lys8-Hβ
	7a		137.5			
	1	10.68		H2	Trp6-C2, Trp6-C7	Trp6-H2, Trp6-H7
	2	7.14	123.7	H1	Trp6-C3, Trp6-C3a, Trp6-C7a	Trp6-H1, Trp6-Hβ
	3		110.1			
	3a		127.9			
Ala7	C=O		170.3			
	NH	5.88		Hα		Trp6-Hα, Ala7-Hβ,
	α	4.05	48.2	NH, Hβ	Ala7-CO, Ala7-Cβ	Ala7-Hβ, Lys8-NH
	β	0.77	20.6	Hα	Ala7-CO, Ala7-Cα	Ala7-Hα, Ala7-NH
Lys8	C=O		170.2			
	NH	7.56		Hα		Lys8-Hα, Lys8-Hβ, Ala7-Hβ
	α	4.05	48.1	NH, Hβ	Lys8-CO	Lys8-Hβ, Lys8-NH, Arg9-NH
	β	2.7	49.6	Hα, Hγ		Trp6-H5, Trp6-H7
	γ	1.75 (Ha) 1.94 (Hb)	28.1	Hβ, Hδ	Lys8-Cδ	Trp6-H7, Lys8-Hβ
	δ	2.29	30.6	Hγ, Hε		Lys8-Hγ (Ha), Lys8-Hγ (Hb)
	ε	3.07	40.8	Hδ, NH (side chain)		Lys8-Hδ
	NH (side chain)	7.73		Hε		
Arg9	C=O		168.7			
	NH	8.23		Hα		
	α	4.09	60.5	NH, Hβ		
	β	2.77 (Ha) 2.82 (Hb)	37.0	Hα, Hγ		

	γ	1.72 (Ha) 1.92 (Hb)	25.4	Hβ, Hδ		
	δ	2.31	30.6	Hγ		
	NH (side chain)	7.51			Arg9-C (guanidine)	
	C (guanidine)		154.4			
Phe10	C=O		172.7			
	NH	8.22		Hα		
	α	4.45	53.9	NH, Hβ		Phe10-Hβ
	β	2.96 (Ha) 3.05(Hb)	29.5	Hα	Phe10-Cα, Phe10-C2, Phe10-C6	Phe10-Hα
	1		137.6			
	2	7.25	129.7	H3	Phe10-Cβ, Phe10-C3, Phe10-C5, Phe10-C6	
	3	7.29	128.9	H2	Phe10-C1, Phe10-C5	
	4	7.23	126.9		Phe10-C2, Phe10-C6	
	5	7.29	128.9	H6	Phe10-C1, Phe10-C3	
	6	7.25	129.7	H5	Phe10-Cβ, Phe10-C3, Phe10-C5, Phe10-C6	

[a]400 MHz in DMSO-d_6 at 298 K. [b]Assigned by HSQC and HMBC. [c]not detected

It will be appreciated that many further modifications and permutations of various aspects of the described embodiments are possible. Accordingly, the described aspects are intended to embrace all such alterations, modifications, and variations that fall within the spirit and scope of the appended claims.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

Throughout this specification and the claims which follow, unless the context requires otherwise, the phrase "consisting essentially of", and variations such as "consists essentially of" will be understood to indicate that the recited element(s) is/are essential

i.e. necessary elements of the invention. The phrase allows for the presence of other non-recited elements which do not materially affect the characteristics of the invention but excludes additional unspecified elements which would affect the basic and novel characteristics of the method defined.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Claims

1. A polypeptide comprising:

a) a first three residue motif (from a N-terminus) and a second three residue motif, the first and second three residue motif optionally separated by 1 to 3 amino acid residue; and

b) at least two C-terminus residues;

wherein the three residue motif is each represented by X_1-X_2-X_3;

wherein each X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine, an unnatural aromatic amino acid residue or a derivative thereof;

wherein each X_2 and X_3 are independently any amino acid residue;

wherein X_1 and X_3 in each motif are connected to form a cyclophane moiety;

wherein at least one of the two C-terminus residues is an aromatic residue.

2. The polypeptide according to claim 1, wherein the first and second three residue motifs are separated by 1 to 3 amino acid residue.

3. The polypeptide according to claim 1 or 2, wherein the first three residue motif is not fused with the second three residue motif via the cyclophane moieties.

4. The polypeptide according to any one of claims 1 to 3, wherein the first X_1 is a residue selected from tryptophan, phenylalanine or a derivative thereof and the second X_1 is a residue selected from phenylalanine, tyrosine or a derivative thereof.

5. The polypeptide according to any one of claims 1 to 43, wherein X_2 is an amino acid residue, the amino acid independently selected from I, G, E, Y, V, L, A, D, S, T, N or Q.

6. The polypeptide according to any one of claims 1 to 5, wherein X_3 is an amino acid residue, the amino acid independently selected from N, R, S, D, Q or K.

7. The polypeptide according to any one of claims 1 to 6, wherein at least one of the two C-terminus residues is a polar and/or basic residue.

8. The polypeptide according to any one of claims 1 to 7, wherein at least one of the two C-terminus residues is an aromatic residue.

9. The polypeptide according to any one of claims 1 to 8, wherein the polypeptide comprises a third three residue motifs.

10. The polypeptide according to any one of claims 1 to 9, wherein when the polypeptide comprises a third three residue motif, X_3 of the first motif and X_1 of the second motif are separated by 1 amino acid residue, and X_3 of the second motif and X_1 of the third motif are covalently bonded to each other via an amide bond.

11. The polypeptide according to any one of claims 1 to 10, wherein the third X_1 is a residue independently selected from tryptophan, phenylalanine or a derivative thereof.

12. The polypeptide according to any one of claims 1 to 11, wherein the polypeptide is represented by Formula (I):

$$X_1\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad (I)$$

wherein each X_1 is an amino acid residue, the amino acid independently selected from tryptophan, phenylalanine, or a derivative thereof;
wherein each X_2 is an amino acid residue, the amino acid independently selected from leucine, isoleucine, valine, alanine, proline, serine, lysine, asparagine, phenylalanine, aspartic acid or a derivative thereof;
wherein each X_3 is an amino acid residue, the amino acid independently selected from lysine, glutamine, asparagine, arginine or a derivative thereof;
wherein X_n is an amide bond or 1 to 3 amino acid residue; and
wherein X_m is at least two C-terminus residues.

13. The polypeptide according to any one of claims 1 to 11, wherein the polypeptide is represented by Formula (II):

$$X_1\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad (II)$$

wherein each X_1 is an amino acid residue, the amino acid independently selected from tryptophan, phenylalanine, tyrosine, or a derivative thereof;
wherein each X_2 is an amino acid residue, the amino acid independently selected from valine, isoleucine, phenylalanine, tryptophan, alanine, leucine, glycine, serine, proline, threonine, aspartic acid, asparagine, glutamic acid, arginine or a derivative thereof;
wherein each X_3 is an amino acid residue, the amino acid independently selected from arginine, lysine, asparagine or a derivative thereof;

wherein X_n is an amide bond or 1 to 3 amino acid residue; and
wherein X_m is at least two C-terminus residues.

14. The polypeptide according to any one of claims 1 to 13, wherein X_1 and X_3 in the second motif are connected via phenylene to form a cyclophane moiety.

15. The polypeptide according to any one of claims 1 to 14, wherein the polypeptide is represented by Formula (Ia), (IIa), (Id) or (IId):

$$W\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad \text{(Ia)}$$

$$W\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad \text{(IIa)}$$

$$F\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad \text{(Id)}$$

$$F\text{-}X_2\text{-}X_3\text{-}X_n\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad \text{(IId).}$$

16. The polypeptide according to any one of claims 1 to 15, wherein the polypeptide is represented by Formula (Ib), (IIb), (Ie) or (IIe):

$$W\text{-}X_2\text{-}X_3\text{-}X_n\text{-}F/Y\text{-}X_2\text{-}X_3\text{-}X_m \quad \text{(Ib)}$$

$$W\text{-}X_2\text{-}X_3\text{-}X_n\text{-}F/Y\text{-}X_2\text{-}X_3\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad \text{(IIb)}$$

$$F\text{-}X_2\text{-}X_3\text{-}X_n\text{-}W\text{-}X_2\text{-}X_3\text{-}X_m \quad \text{(Ie)}$$

$$F\text{-}X_2\text{-}X_3\text{-}X_n\text{-}W\text{-}X_2\text{-}X_3\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_m \quad \text{(IIe).}$$

17. The polypeptide according to any one of claims 1 to 16, wherein when X_1 is W, X_1 is connected to X_3 via a 3,6 or 3,7 substituted indolylene moiety.

18. The polypeptide according to any one of claims 1 to 17, wherein when X_1 is F or Y, X_1 is connected to X_3 via a 1,3 or 1,4 disubstituted phenylene moiety.

19. The polypeptide according to any one of claims 1 to 18, wherein the polypeptide is represented by Formula (IIc):

$$W\text{-}X_2\text{-}X_3\text{-}X_n\text{-}F/Y\text{-}X_2\text{-}X_3\text{-}W\text{-}X_2\text{-}X_3\text{-}X_m \quad \text{(IIc).}$$

20. The polypeptide according to any one of claims 1 to 19, wherein the polypeptide is selected from:

WVNA**F**AN**W**TKRF (SEQ ID 19)
WVNA**F**AN**W**PKRF (SEQ ID 17)
WINA**F**AN**W**TKRI (SEQ ID 13)

WWRA**Y**AR**W**RRSF (SEQ ID 37)
WVNA**F**AR**W**GKSF (SEQ ID 4)
G**W**FRA**Y**LR**W**SRSF (SEQ ID 36)
WVNA**Y**AR**W**TNRF (SEQ ID 25)
WVNA**F**AK**W**TKRI (SEQ ID 14)
WVNA**Y**AR**W**TKRF (SEQ ID 26)
WVNV**F**AR**W**DKQI (SEQ ID 22)
WVNF**F**AK**F**TKSF (SEQ ID 15)
WVNA**F**AR**W**SRRW (SEQ ID 30)
WVNA**F**AR**W**SKSF (SEQ ID 8)
WVNV**F**AR**W**SRRW (SEQ ID 34)
AG**W**IRA**F**AN**W**SRSF (SEQ ID 35)
WVNA**F**AR**W**DKKF (SEQ ID 23)
WVNA**F**AR**F**TKRF (SEQ ID 20)
WVNV**F**AR**W**DKAI (SEQ ID 10)
WLNV**F**VR**W**DRAI (SEQ ID 24)
WINV**F**AR**W**NRAI (SEQ ID 21)
WINA**F**GN**W**ERAFH (SEQ ID 32)
WVNA**F**AN**W**SKSF (SEQ ID 3)
WVNA**F**AN**W**SKAL (SEQ ID 1)
WVNA**F**GN**W**SKSL (SEQ ID 2)
WVNA**F**LN**W**SRSF (SEQ ID 16)
WVNA**F**LR**W**GKSF (SEQ ID 12)
WINA**F**AR**W**GRAF (SEQ ID 7)
AG**W**IKV**F**GN**W**SRSF (SEQ ID 33)
WVNA**F**VN**W**TKSF (SEQ ID 9)
WVNA**F**LN**W**PRSF (SEQ ID 18)
AG**W**IKA**F**GN**W**SRSF (SEQ ID 29)
WVNA**F**VN**W**PKSF (SEQ ID 6)
AG**W**INA**F**AN**W**TKSF (SEQ ID 28)
AG**W**INA**F**AN**W**TRSF (SEQ ID 31)
AG**W**INA**F**GN**W**TKSF (SEQ ID 27)
WVNA**F**AR**W**GRAF (SEQ ID 5)
WVNA**F**AR**W**SKRW (SEQ ID 38)
WVNA**F**AR**W**SKRF (SEQ ID 39)

RGEG**W**VRAY**W**AKRF (SEQ ID 50)
KPGEG**W**VNFT**W**NKSF (SEQ ID 52)
KSEAAGG**W**VNFQ**W**KNSW (SEQ ID 46)
AGNDG**W**VKFG**W**KKKF (SEQ ID 49)
ASTAET**W**FKLD**W**KKSF (SEQ ID 54)
DGR**W**LQ**W**IKNH (SEQ ID 41)
GDR**W**LK**W**IKNH (SEQ ID 40)
VGG**F**ANAT**W**SKSF (SEQ ID 44)
VGG**F**ANAS**W**PKSF (SEQ ID 43)
VGG**F**ANAT**W**PKSF (SEQ ID 45)
NA**F**VNAT**W**SRAM (SEQ ID 59)
NV**F**VNATWSRAM (SEQ ID 47)
NV**F**VNAT**W**SRAI (SEQ ID 60)
SSDDDGI**F**FKTT**W**DRR (SEQ ID 55)

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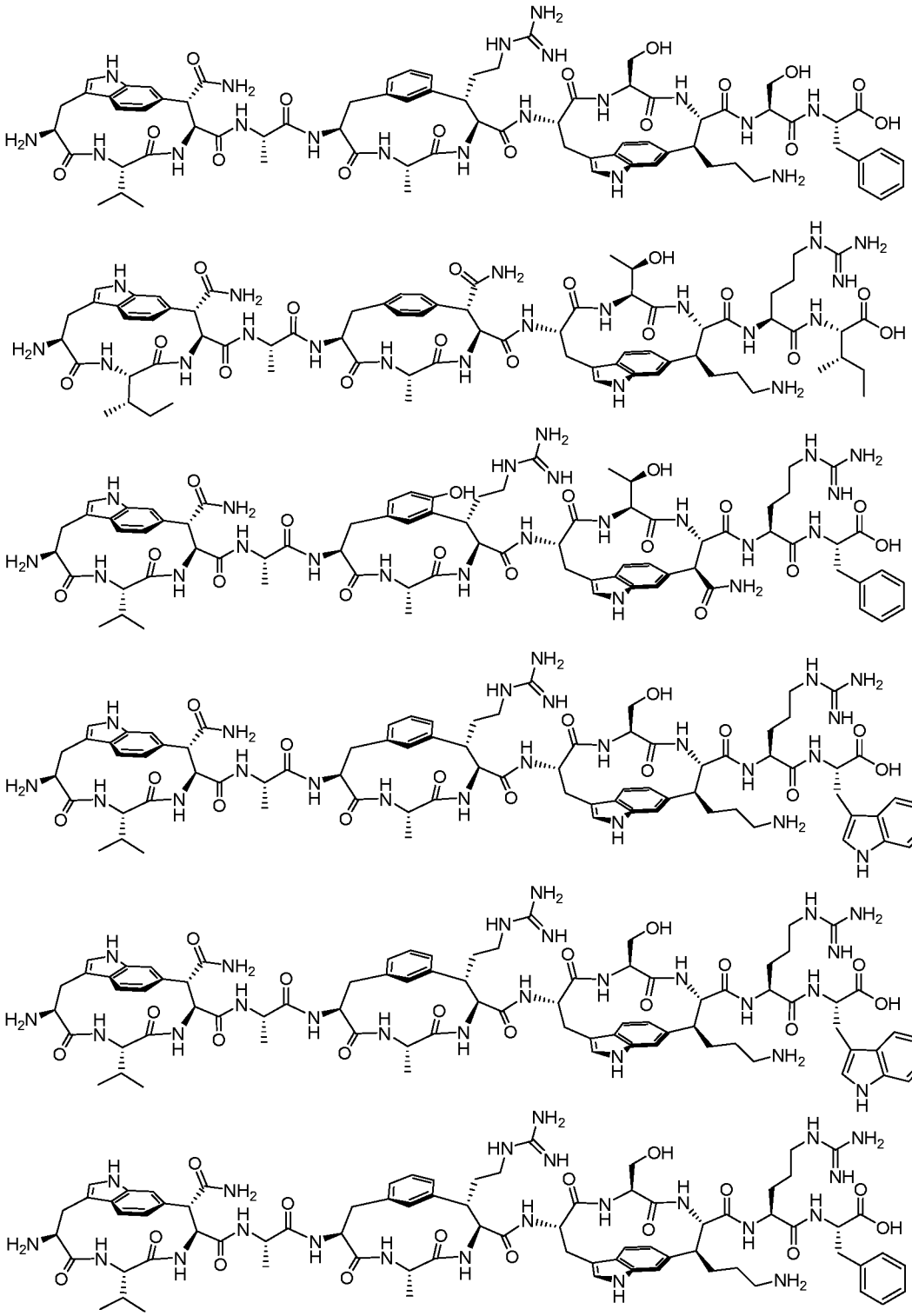
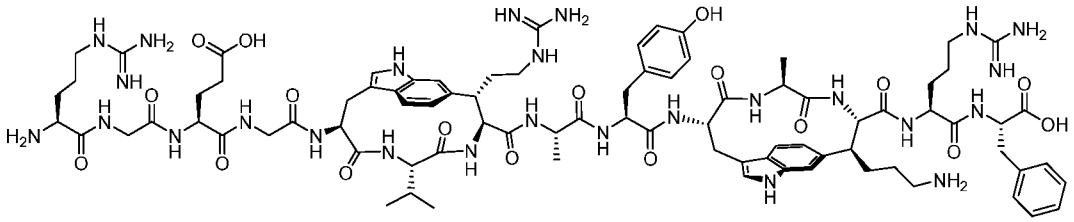
21. The polypeptide according to any one of claims 1 to 20, wherein the polypeptide is selected from:





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22. The polypeptide according to any one of claims 1 to 21, wherein the polypeptide is an isolated polypeptide.

23. The polypeptide according to any one of claims 1 to 22, wherein the polypeptide is characterised by an antibacterial activity.

24. The polypeptide according to any one of claims 1 to 23, wherein the polypeptide is characterised by a minimal inhibitory concentration (MIC) of about 2 μg/mL to about 10 μg/mL.

25. A composition comprising a polypeptide according to any one of claims 1 to 24.

26. A method of producing a polypeptide in a host cell, the method comprising:
a) introducing to the host cell one or more nucleic acid molecules, the nucleic acid moleucles configured to express a precursor polypeptide (A), a rSAM/SPASM maturase (B), a protease (C), a transporter (D) and a protease/transporter (E);
wherein the precursor polypeptide comprises a first three residue motif (from a N-terminus) and a second three residue motif, the first and second three residue motif optionally separated by 1 to 3 amino acid residue, and at least two C-terminus residues;
wherein the three residue motif is each represented by X_1-X_2-X_3;
wherein each X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine, an unnatural aromatic amino acid residue or a derivative thereof;
wherein each X_2 and X_3 are independently any amino acid residue;
wherein at least one of the two C-terminus residues is an aromatic residue;
wherein the rSAM/SPASM maturase is capable of modifying the precursor polypeptide in the host cell to form a modified precursor polypeptide with a cyclophane moiety connecting the X_1 and X_3 residues in each motif;
wherein the protease, transporter and protease/transporter are capable of cleaving the

modified precursor polypeptide from the rSAM/SPASM maturase to form a cleaved modified polypeptide and exporting the cleaved modified polypeptide out from the host cell.

27. The method according to claim 26, wherein at least the nucleic acid moleucle configured to express A is derived from a Xye maturase system.

28. The method according to claim 26 or 27, wherein the nucleic acid moleucles configured to express A and B are from one Xye species and the nucleic acid moleucles configured to express C, D and E are from another Xye species.

29. The method according to any one of claims 26 to 28, wherein at least the nucleic acid moleucles configured to express C, D and E are fused.

30. The method according to any one of claims 26 to 29, wherein the nucleic acid moleucles configured to express A and B are fused.

31. The method according to claim 26 or 27, wherein the nucleic acid moleucles configured to express B, C, D and E are fused.

32. The method according to any one of claims 26 to 31, wherein the nucleic acid moleucles configured to express A, B, C, D and E are fused.

33. The method according to any one of claims 26 to 32, wherein the nucleic acid moleucle configured to express A is at least 70% identical to and derived from a bacterial species selected from *Serratia marcescens (smc)*, *Erwinia toletana (etc)*, *Photorhabdus australis (pac), Xenorhabdus nematophila (xnc), Xenorhabdus griffiniae VH1 (xgc), Pandoraea sp. PE-S2R-1 (psc), Pandoraea oxalativorans DSM 23570 (poc), Photorhabdus heterorhabditis Q614 (phc), Kosakonia cowanii pasteuri (kcc2 and kcc1), Bordetella bronchialis AU17976 (bbc)* and *Photorhabdus laumondii BOJ-47 (plc).*

34. The method according to any one of claims 26 to 32, wherein the nucleic acid moleucles configured to express C, D and E are at least 70% identical to and derived from *Xenorhabdus nematophila (xnc).*

35. The method according to any one of claims 26 to 34, wherein the rSAM/SPASM maturase has an amino acid sequence that is at least 70% identical to one of the following:

XncB:

MTTSKSEKIKHLEIILKISERCNINCSYCYVFNMGNSLATDSPPVISLDNVLALRGFFERSAAENEI
EVIQVDFHGGEPLMMKKDRFDQMCDILRQGDYSGSRLELALQTNGILIDDEWISLFEKHKVHASI
SIDGPKHINDRYRLDRKGKSTYEGTIHGLRMLQNAWKQGRLPGEPGILSVANPTANGAEIYHHFA
NVLKCQHFDFLIPDAHHDDDIDGIGIGRFMNEALDAWFADGRSEIFVRIFNTYLGTMLSNQFYRV
IGMSANVESAYAFTVTADGLLRIDDTLRSTSDEIFNAIGHLSELSLSGVLNSPNVKEYLSLNSELPS
DCADCVWNKICHGGRLVNRFSRANRFNNKTVFCSSMRLFLSRAASHLITAGIDEETIMKNIQK
(SEQ ID NO: 61)

YkcB:

MEVITGSEGRVMLNLLIEKNIRHLEIILKISERCNINCDYCYVFNKGNSAADDSPARLSNKNIHHLV
CFLQRACQEYKIGTVQIDFHGGEPLLMKKENFTDMCIQLISGNYCGSNIRLALQTNATLIDNEWIA
IFEKYSVNVSISIDGPKHINDRHRLDTKGRSTYESTVRGLRILQNAYQQGRLPSDPGILCVTNAQA
NGAEIYRHFVDELGVYSFDFLIPDDSYKDAHPDAVGIGRFLNEALDEWVKDNNAKIFVRLFQTHIA
SLLGQKNSGVLGHTPNITGVYALTVSSDGFVRVDDTLRSTSDRMFNPIGHLSEVNLSNVFASPQF
QEYSSIGQSLPTECEGCIWENICAGGRIVNRFSTEDRFKHKSIYCYSMRTFLSRSSAHLLNMGIKE
ERIMAAIRA (SEQ ID NO: 62)

EtcB

MTQLKGEKIKHLEIILKISERCNINCTYCYVFNMGNTLATDSTPVISLDNVYALRGFFERSAAENDI
EVIQVDFHGGEPLMMKKDRFDRMCQILLQGNYRSSKFELALQTNGILIDDEWIALFEKHQVHASI
SVDGPKHINDRHRLDRKGKSTYEGTITGLRLLQNAWQQGRLPGEPGILSVANANANGAEIYRHF
ADTLQCQRFDFLIPDDHHDDSPDGEGVGRFLNEALDAWFADGRPEIFIRIFNTYLGTMLNSQFNR
VLGMSANVESAYAFTVTADGMLRIDDTLRSTSDEIFNAVGHVSELSLARVLETSCVKEYLALSSNL
PTVCAECVWNNICHGGRLVNRFSRTNRFNNKTVFCKSMRLFLSRAASHLMASGVDEKEIMKNIQ
K (SEQ ID NO: 63)

MscB

MAPGPARAALTEFVLKVHARCDLACDHCYVYEHADQSWRRRPVRMTPEVLRTAAGRIAEHAAAH
DLPDVTVILHGGEPLLLGAERLGEVLADLRRVIDPVTRLRLGMQTNGVLLSERLCDLLAEHDVAVG
VSLDGDRAANDRHRRFRSGAGSYDQVLRAIGLLRRPAYRRIYSGLLCTVDVRNDPIAVYESLLTQ
EPPRIDFLLPHATWDDPPWRPAGGGTAYAGWLRAVYDRWLADGRPVSVRLFDSLLSTAAGGPS

GTEWLGLDPVDLAVVETDGEWEQADSLKTAYDGAPATGMTVFSHAADDVAASPLLARRRSGRA
GLSDECRRCPVVDQCGGGLFAHRYGAGHFDHPSVYCADLKELIVHVNENPPAPVRLDAGLPDDF
IDRLAALTGDRVAIGRLVEAQIAIVRALLAEVADRLPAGGAGADGWEALTALDRSAPESVARIAAH
PYVRAWAVDCLAGSGTGARQGPDYLSALAVAAALDAGTPVRLDVPVRSGRLHLPTVGTVLLPEV
GDGAARVETGPGSLRVAAGDVTVAIRPGTPGDAPRWWPTRVLAAPDVSVLLEDGDPHRDCHRL
PAGDRLDDAGAARWAETFAAAWQVIRDEVPGHAEELRAGLRAVVPLRRSGAGVSEASTARQAF
GGVAATETDAGSLAVLLVHEFQHSKMNALLDICDLVDGTRPIDITVGWRPDPRPAEAVLHGIYAH
AAVADIWRIRADRQVDGAQAVYRRYRDWTAEAIGALQRADALTPAGSRLVRQVARSMSGWPS
(SEQ ID NO: 64)

OscB:
MINPTLLNPEKIDISKFGPINLVVIQATSFCNLNCDYCYLPNRDLKNTLSLDLIEPIFKNIFNSPFVG
DEFTICWHAGEPLAVPISFYESAFQLIQAADQKYNQKQAKIWHSVQTNATYINQKWCDFIQEHNI
CVGVSLDGPEFIHDAHRQTRKGTGSHAQTMRGISFLQKNNIPFYVISVVTQDSLNYADEIFNFFR
ENGIYDVGFNLEEIEGVNQSSTLEAVGTSEKYRAFMQRFWELTSEVQGEFNLREFEAICGLIYSNT
RLTQTDMNNPFVLINIDYQGNFSTFDPELLSVNIKPYGNFILGNVLTDSFESVCDTEKFQKIYTDM
QEGIKLCRETCEYFGVCGGGAGSNKYWENGTFACSETMACRYRIKVVTDIILDKLENSLGLVENC
(SEQ ID NO: 65)

LscB:
MTISKMNLPVQTDNFRASSTLDLSAFGPINLVVIQSTSFCNLNCDYCYLRDRQSKNRLSLDLIEPIL
KTVLTSPFVGCDFTILWHAGEPLAMPISFYDSATALIREAERQYKTQPIQIFQSIQTNATLINQAWC
DCFRRNEIYVGVSLDGPAFLHDAHRQTYKGTGTHAATMRGISLLQKNEIPFNVICVLTQDSLDYP
DEIFNFFRSNRITEVGFNMEEAEGVHQHSTLDQQGTEERYRAFMQRFWDLTVQAKGEFKLREFE
TICTLAYTGDRLGYTDMNQPFVIVNFDHQGNFSTFDPELLSFKIKEYGDFVLGNVLHNTLESVCQT
EKFQKIYQDMAAGVVQCRQSCEYFGLCGGGAGSNKYWENGTFNCTETKACRYRIKVIADIVLEG
LENSLELANSIS (SEQ ID NO: 66)

GscB
MSIVTSKPVINFKNTANFGPISLIIIQPNSFCNLDCDYCYLPDRHLQNKLSLDLIDPIFKSIFTSPFLG
CDFGVCWHAGEPLTMPVSFYKSAFQLIEEANTKYNKSEYSFYHSYQTNGTLINQGWCDLWQEYP
VHVGVSIDGPAFLHDVHRKNRKGGNSHDLTMRGIRYLQKNNIPYNTISVITEESLNYPDEMFNFF
AENEIYDLAFNMEETEGVNELTSLNGIEIEHKYSQFIKRFWQLVTESKLPFIVREFEILISLIYSGNR
LTNTDMNKPFVIVNFDYQGNFSTFDPELLSVKTDKYGDFIFGNVLKDSLESICETEKFKTIYKDIND

GVKLCSDNCSYFGICGGGAGSNKYWENGTFASMETQACRYRIKILTDVLVSTIENSLGL (SEQ ID NO: 67)

MscB-375
MAPGPARAALTEFVLKVHARCDLACDHCYVYEHADQSWRRRPVRMTPEVLRTAAGRIAEHAAAH DLPDVTVILHGGEPLLLGAERLGEVLADLRRVIDPVTRLRLGMQTNGVLLSERLCDLLAEHDVAVG VSLDGDRAANDRHRRFRSGAGSYDQVLRAIGLLRRPAYRRIYSGLLCTVDVRNDPIAVYESLLTQ EPPRIDFLLPHATWDDPPWRPAGGGTAYAGWLRAVYDRWLADGRPVSVRLFDSLLSTAAGGPS GTEWLGLDPVDLAVVETDGEWEQADSLKTAYDGAPATGMTVFSHAADDVAASPLLARRRSGRA GLSDECRRCPVVDQCGGGLFAHRYGAGHFDHPSVYCADLKELIVHVNENPPAPV (SEQ ID NO: 68).

36. The method according to any one of claims 26 to 35, wherein the rSAM/SPASM maturase is characterised by a rSAM domain and a SPASM domain;
wherein the rSAM domain is CNINCSYC (SEQ ID NO: 69); and
wherein the SPASM domain is CADCVWNKIC (SEQ ID NO: 70).

37. The method according to any one of claims 26 to 36, wherein the nucleic acid molecules are introduced into the host cell via a pET28a(+) vector, pCDFduet-1 vector, pACYCDuet-1 vector, pETDuet-1 vector, pCOLADuet-1 vector, pRSFDuet-1 vector, pBAD vector, or a combination thereof.

38. The method according to any one of claims 26 to 37, wherein the host cell is E. coli NiCo21(DE3), BL21(DE3), BL21-AI, BL21 Star™ (DE3) pLysS, Rosetta™(DE3), or a combination thereof.

39. A method of producing a polypeptide, the method comprising:
a) expressing a precursor polypeptide and a rSAM/SPASM maturase; wherein the precursor polypeptide comprises a first three residue motif (from a N-terminus) and a second three residue motif, the first and second three residue motif optionally separated by 1 to 3 amino acid residue, and at least two C-terminus residues;
wherein the three residue motif is each represented by X_1-X_2-X_3;
wherein each X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine, an unnatural aromatic amino acid residue or a derivative thereof;
wherein each X_2 and X_3 are independently any amino acid residue;

wherein at least one of the two C-terminus residues is an aromatic residue;
wherein the rSAM/SPASM maturase is capable of modifying the precursor polypeptide to form a polypeptide with a cyclophane moiety connecting the X_1 and X_3 residues in each motif.

40. A method of synthesising a polypeptide according to any one of claims 1 to 24, the method comprising:
(a) coupling a pre-sequence peptide to a support, wherein said pre-sequence peptide comprises amino acid residues having side chain functionalities which are, if necessary, protected during the synthesis;
(b) coupling one or more N-protected amino acids to the N-terminus of the pre-sequence peptide to form a precursor polypeptide, wherein each coupling is performed in stepwise fashion and under conditions in which each of the amino acids of the target peptide is coupled and subsequently N-deprotected;
c) cleaving said precursor polypeptide from the support; and
d) synthetically or enzymatically connecting the X_1 and X_3 in each motif to form a cyclophane moiety.

41. A method of modifying a precursor polypeptide, the precursor polypeptide comprising:
a) a first three residue motif (from a N-terminus) and a second three residue motif, the first and second three residue motif optionally separated by 1 to 3 amino acid residue; and
b) at least two C-terminus residues;
wherein the three residue motif is each represented by X_1-X_2-X_3;
wherein each X_1 is a residue independently selected from tryptophan, phenylalanine, tyrosine, histidine, an unnatural aromatic amino acid residue or a derivative thereof;
wherein each X_2 and X_3 are independently any amino acid residue; and
wherein at least one of the two C-terminus residues is an aromatic residue;
the method comprising:
enzymatically connecting the X_1 and X_3 residues in each motif to form a cyclophane moiety.

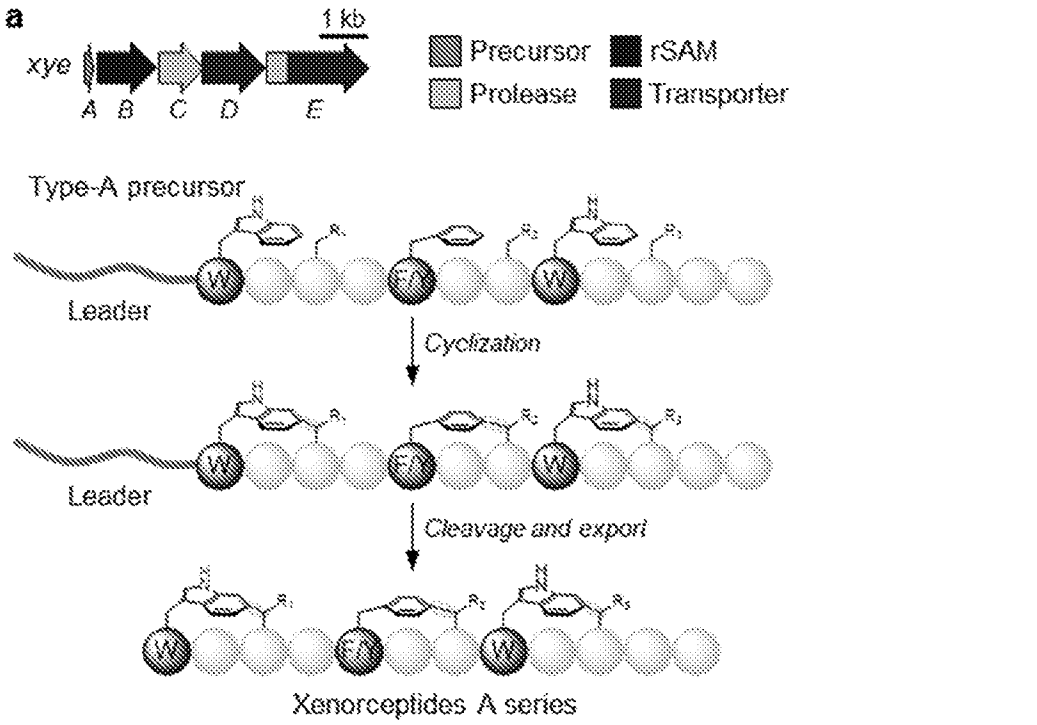
42. The method according to claim 41, wherein the enzyme is rSAM/SPASM maturase.

43. A method of treating a bacterial infection in a subject in need thereof, comprising administering an effective amount of a polypeptide according to any one of claims 1 to 24 to the subject.

44. The method according to claim 43, wherein the bacterial infection is a Gram-negative bacterial infection.

45. The method according to claim 43 or 44, wherein the bacterial infection is characterised by a drug-resistance.

46. The method according to any one of claims 43 to 45, wherein the bacterial infection is caused by a Gram-negative bacteria selected from *Escherichiacoli, Pseudomonas aeruginosa, Candidatus Liberibacter, Agrobacterium tumefaciens, Acinetobactor baumannii, Moraxella catarrhalis, Citrobacter di versus, Enterobacter aerogenes, Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhimurium, Neisseria meningitidis, Serratia marcescens, Shigella sonnei, Shigella boydii, Neisseria gonorrhoeae, Acinetobacter baumannii, Salmonella enteriditis, Fusobacterium nucleatum, Veillonella parvula, Actinobacillus actinomycetemcomitans, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Helicobacter pylori, Francisella tularensis, Yersinia pestis, Vibrio cholera, Morganella morganii, Edwardsiella tarda, Campylobacter jejuni, Haemophilus influenza, Enterobacter cloacae*, or a combination thereof.



b

Type A precursor…GG**WVNAFARWSK**SF

Xenorceptide A2 **WVNAFARWSK**SF (SEQ ID 8)

ΩxxxΩxxΩxx

Type B precursor …GGDR**WLKWIK**NH

Xenorceptide B1 DR**WLKWIK**NH (SEQ ID 40)

ΩxxΩxx

Type C Precursor …GG**WVNAFLN**

(SEQ ID 42) **WVNAFLN**

ΩxxxΩxx

Type D Precursor

…GGRGEG**WVR**AY**WAK**RF

Xenorceptide D1 RGEG**WVR**AY**WAK**RF (SEQ ID 50)

ΩxxxxΩxx

Figure 1

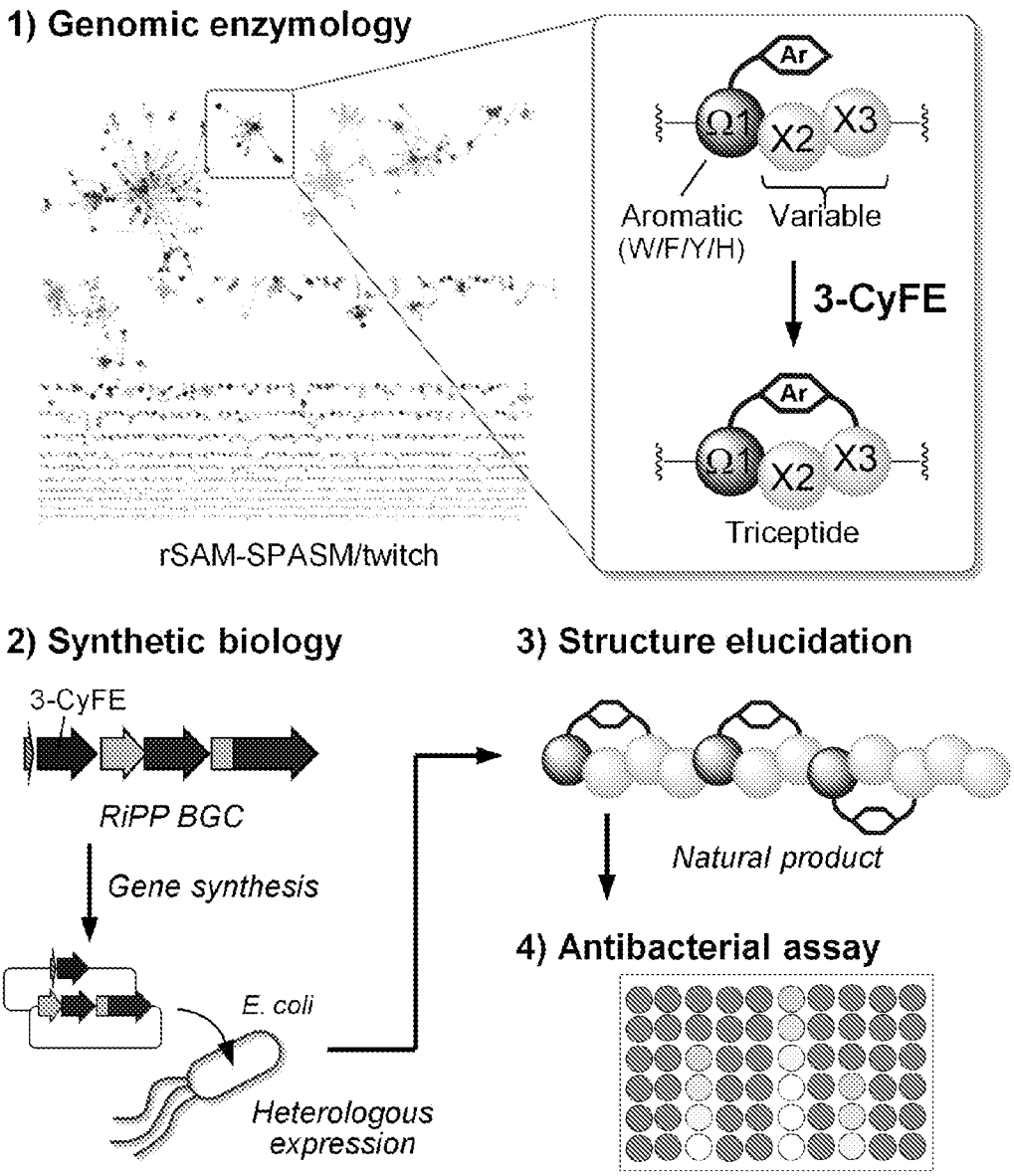

1) Genomic enzymology
rSAM-SPASM/twitch
Ar
Q1
X2
X3
Aromatic
(W/F/Y/H)
Variable
3-CyFE
Triceptide
2) Synthetic biology
3-CyFE
RiPP BGC
Gene synthesis
E. coli
Heterologous
expression
3) Structure elucidation
Natural product
4) Antibacterial assay

Figure 2

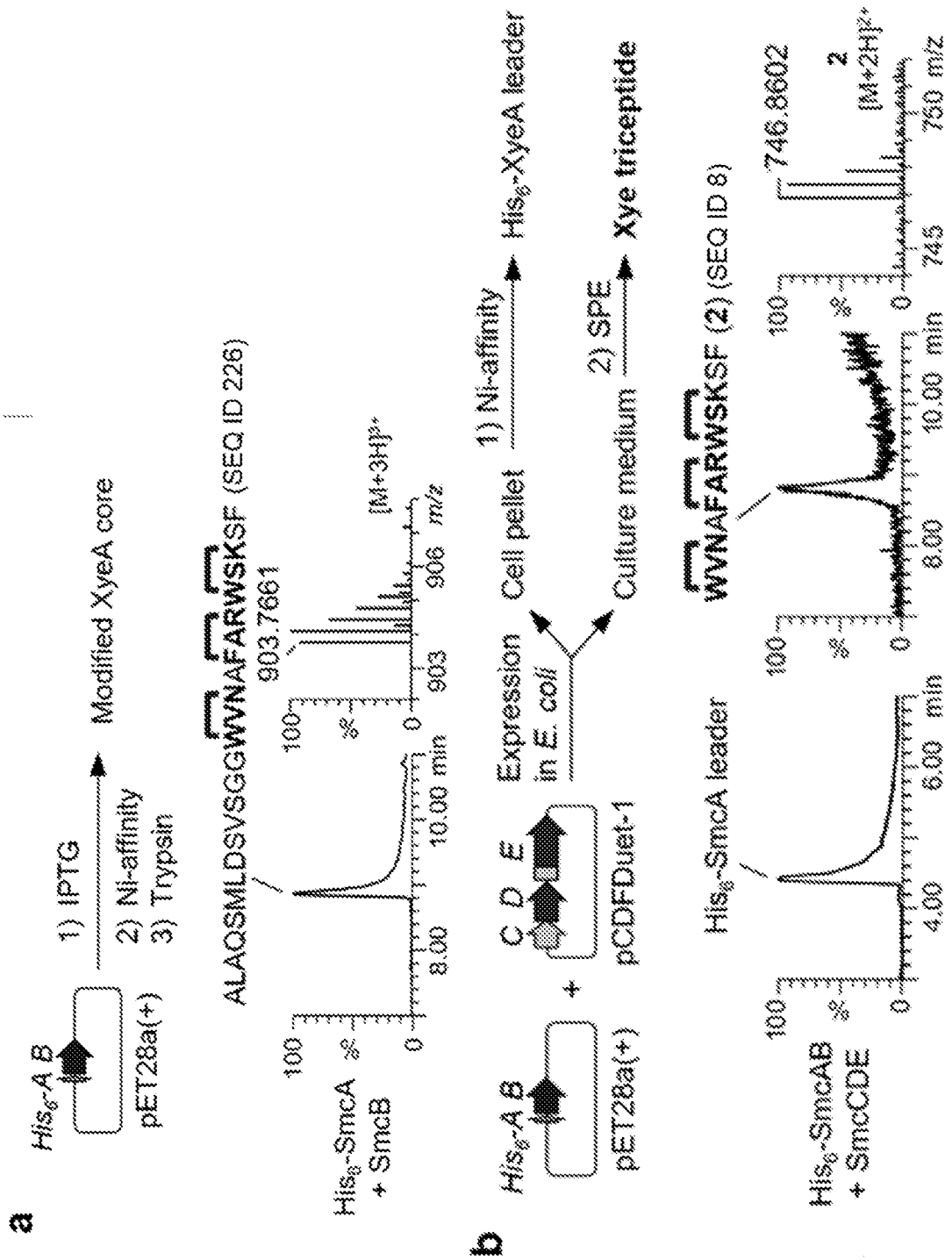

a
His6-A B
pET28a(+)
1) IPTG
2) Ni-affinity
3) Trypsin
Modified XyeA core
His6-SmcA + SmcB
ALAQSMLDSVSGGWVNAFARWSKSF (SEQ ID 226)
903.7661
[M+3H]3+
903
906 m/z
8.00
10.00 min
b
His6-A B
pET28a(+)
C D E
pCDFDuet-1
Expression in E. coli
Cell pellet
Culture medium
1) Ni-affinity
2) SPE
His6-XyeA leader
Xye triceptide
His6-SmcA leader
WVNAFARWSKSF (2) (SEQ ID 8)
His6-SmcAB + SmcCDE
746.8602
2
[M+2H]2+
745
750 m/z
4.00
6.00 min
8.00
10.00 min

Figure 3a-b

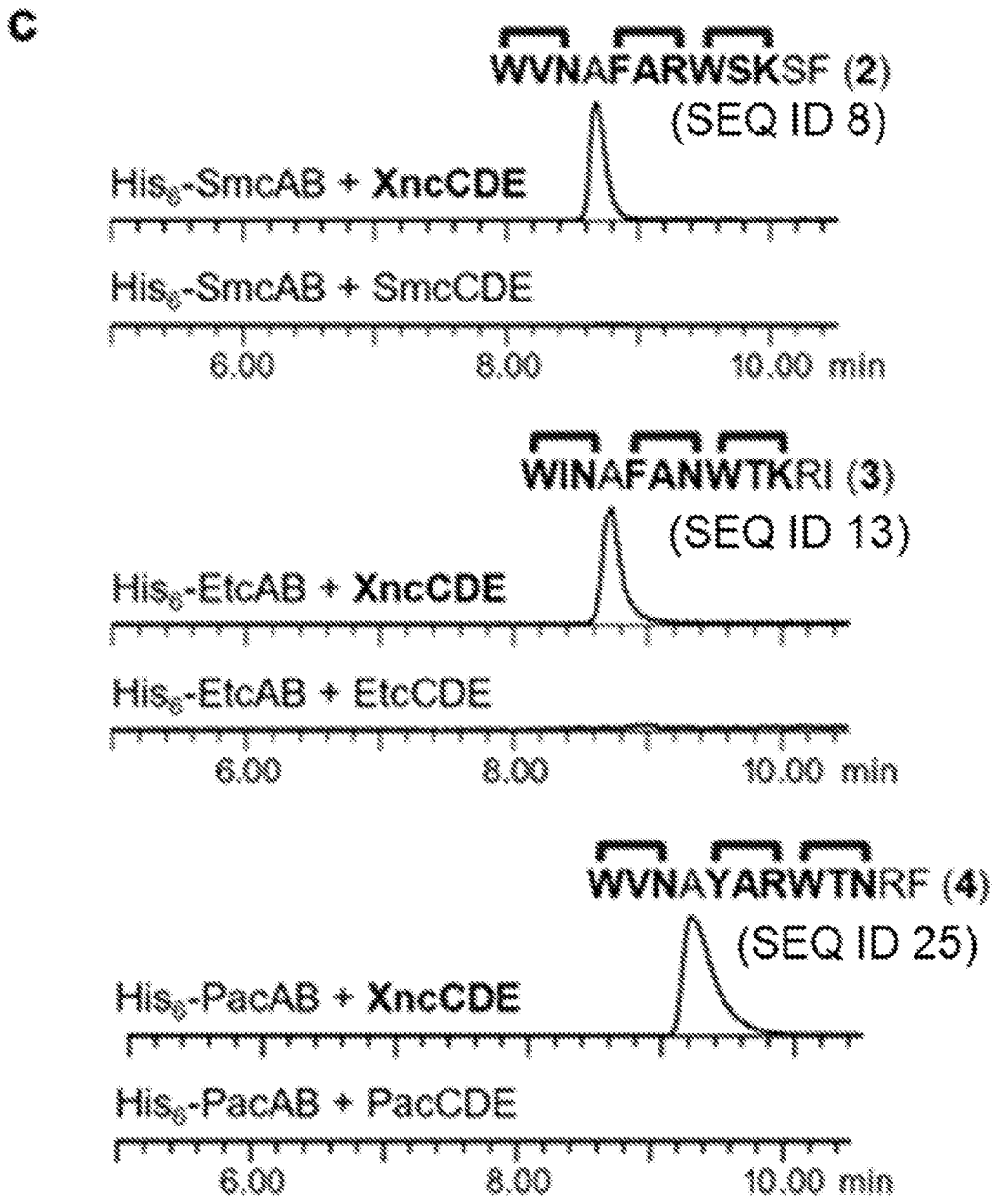

c
WVNAFARWSKSF (2)
(SEQ ID 8)
His6-SmcAB + XncCDE
His6-SmcAB + SmcCDE
6.00
8.00
10.00 min
WINAFANWTKRI (3)
(SEQ ID 13)
His6-EtcAB + XncCDE
His6-EtcAB + EtcCDE
6.00
8.00
10.00 min
WVNAYARWTNRF (4)
(SEQ ID 25)
His6-PacAB + XncCDE
His6-PacAB + PacCDE
6.00
8.00
10.00 min

Figure 3c

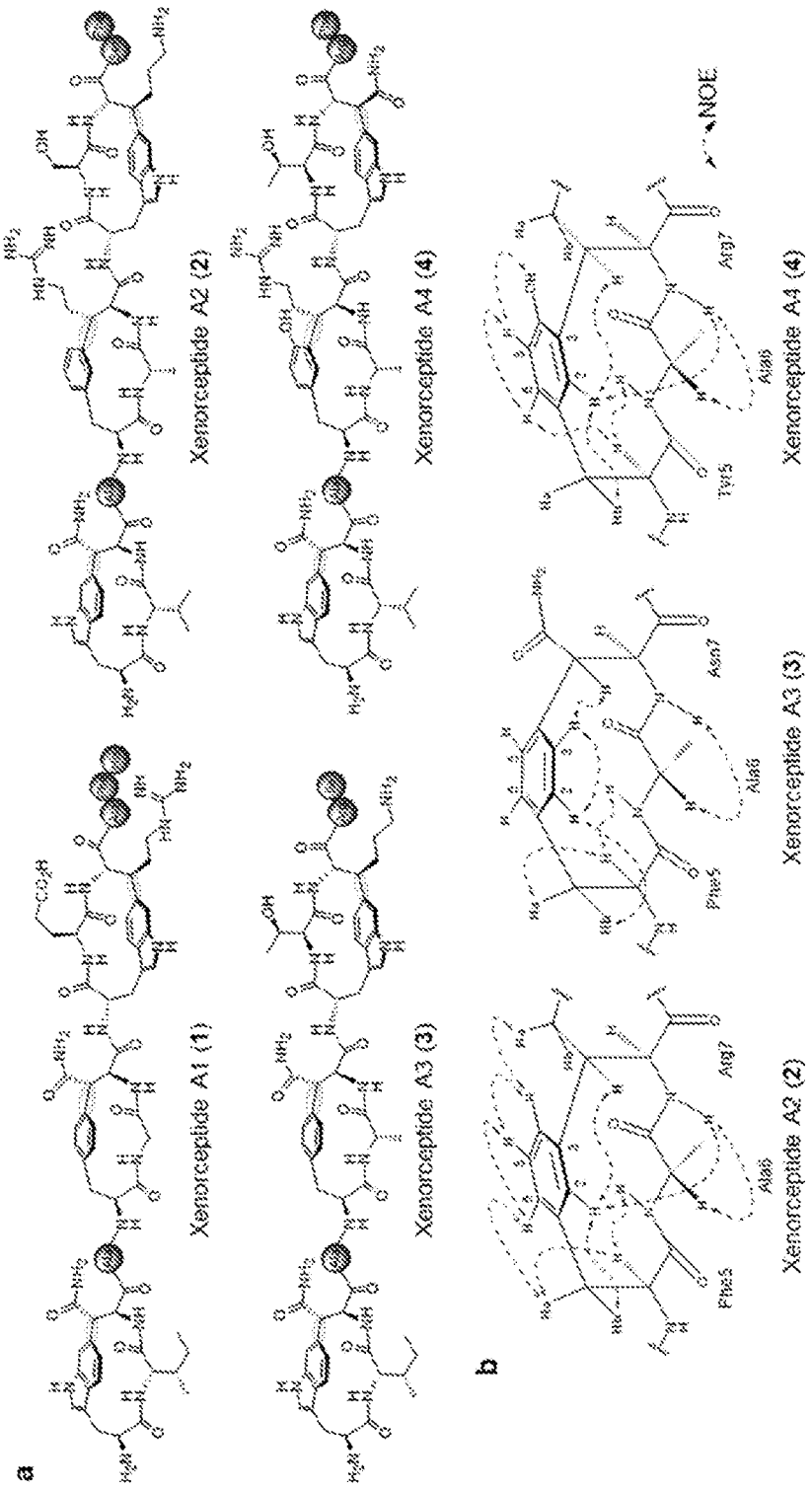

a
Xenorceptide A1 (1)
Xenorceptide A2 (2)
Xenorceptide A3 (3)
Xenorceptide A4 (4)
b
Xenorceptide A2 (2)
Xenorceptide A3 (3)
Xenorceptide A4 (4)
Phe5
Ala6
Arg7
Asn7
Trp5
NOE

Figure 4

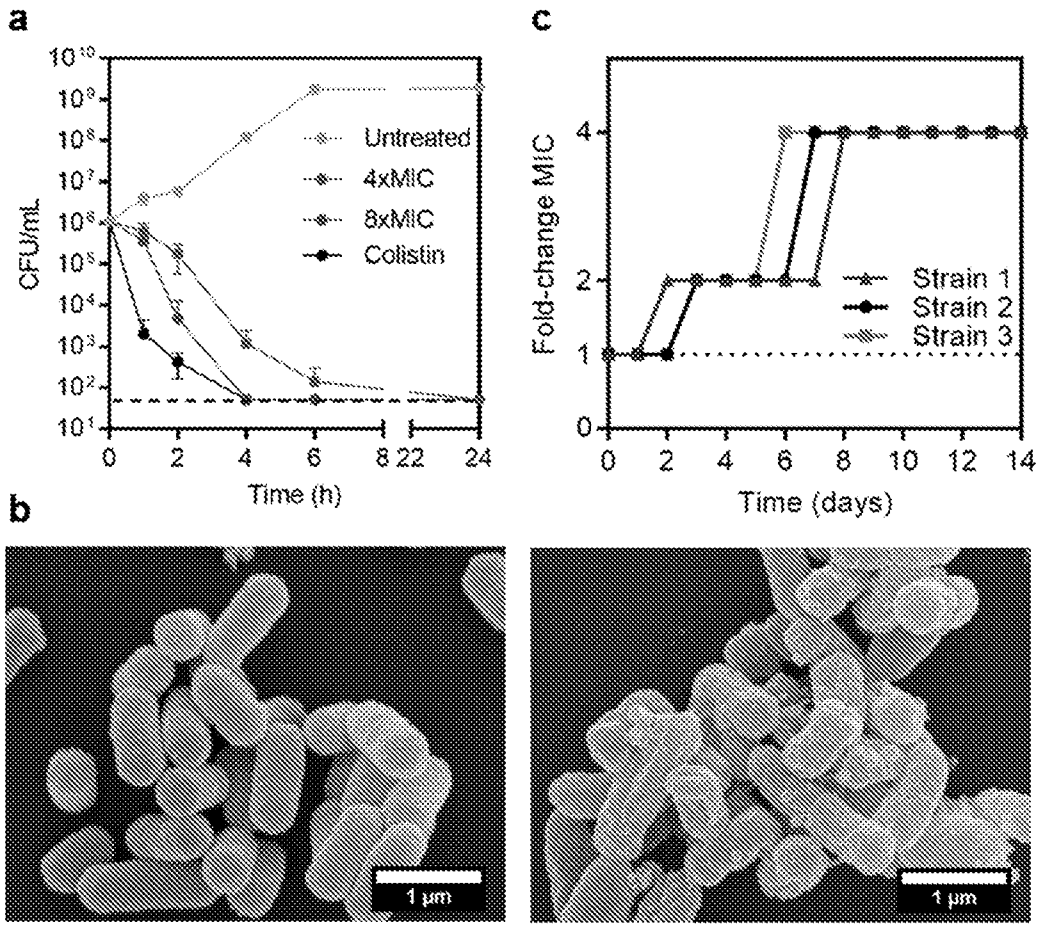

a
c
b
CFU/mL
Untreated
4xMIC
8xMIC
Colistin
Time (h)
Fold-change MIC
Strain 1
Strain 2
Strain 3
Time (days)
1 µm
1 µm

Figure 5

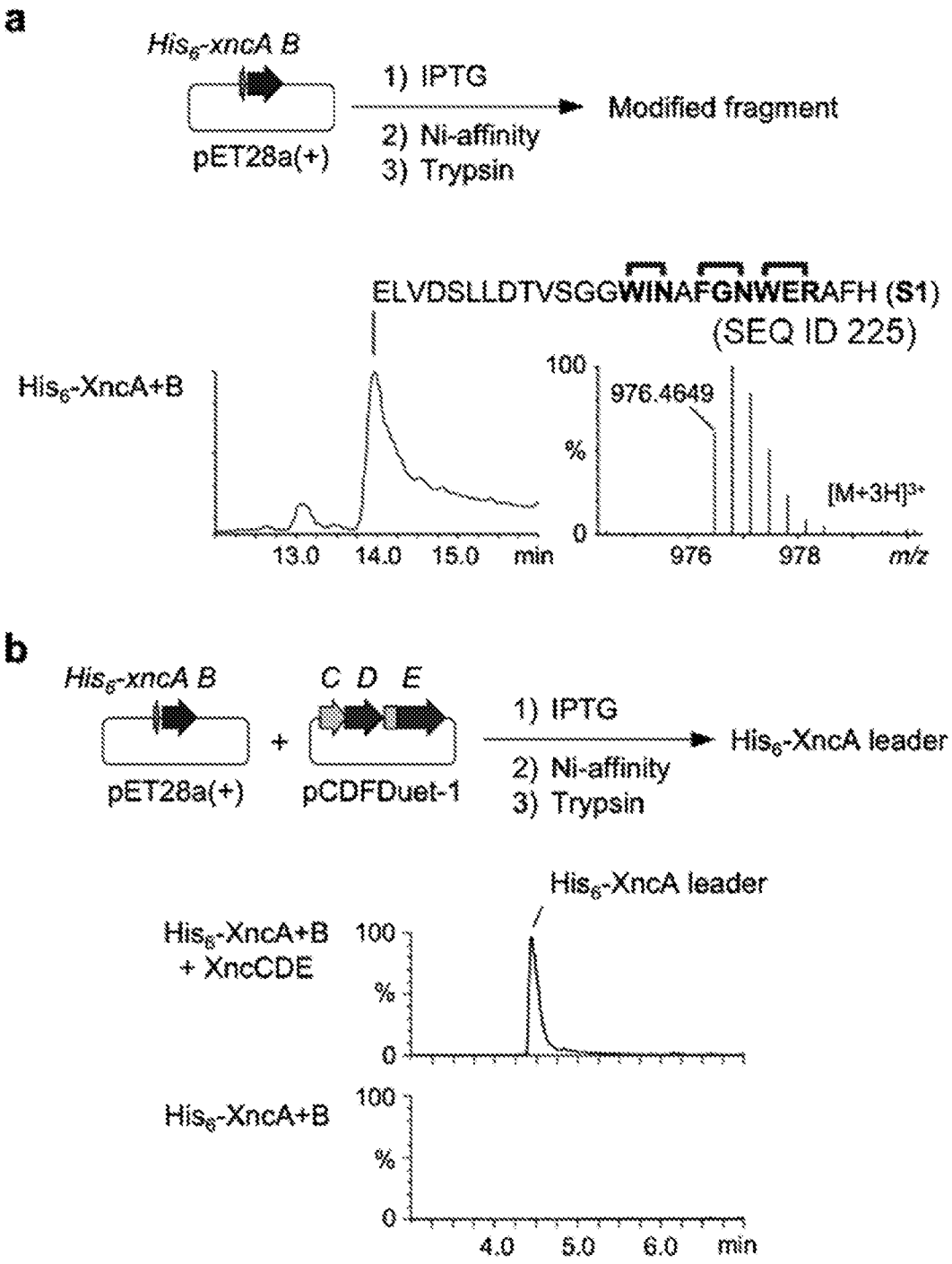

a
His6-xncA B
pET28a(+)
1) IPTG
2) Ni-affinity
3) Trypsin
Modified fragment
ELVDSLLDTVSGGWINAFGNWERAFH (S1)
(SEQ ID 225)
His6-XncA+B
100
%
0
976.4649
[M+3H]3+
13.0
14.0
15.0
min
976
978
m/z
b
His6-xncA B
C D E
pET28a(+)
pCDFDuet-1
1) IPTG
2) Ni-affinity
3) Trypsin
His6-XncA leader
His6-XncA+B
+ XncCDE
His6-XncA leader
His6-XncA+B
4.0
5.0
6.0
min

Figure 6

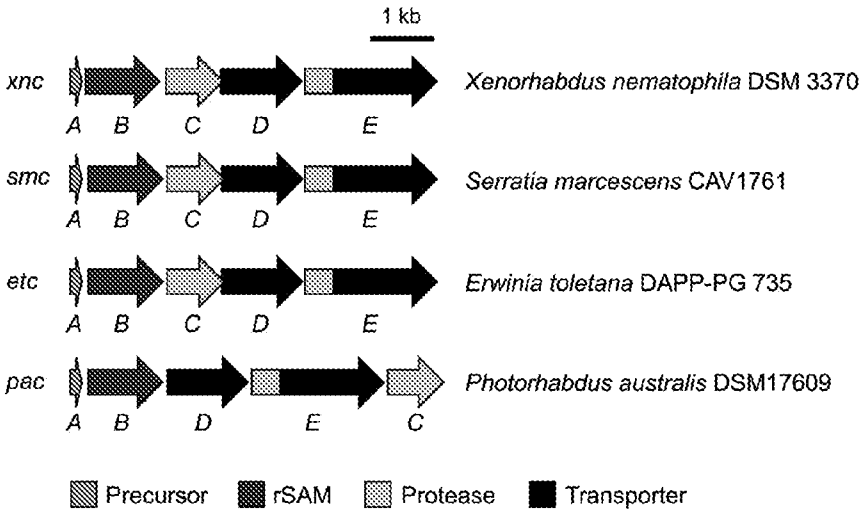

1 kb
xnc
A B C D E
Xenorhabdus nematophila DSM 3370
smc
A B C D E
Serratia marcescens CAV1761
etc
A B C D E
Erwinia toletana DAPP-PG 735
pac
A B D E C
Photorhabdus australis DSM17609
Precursor
rSAM
Protease
Transporter

Figure 7

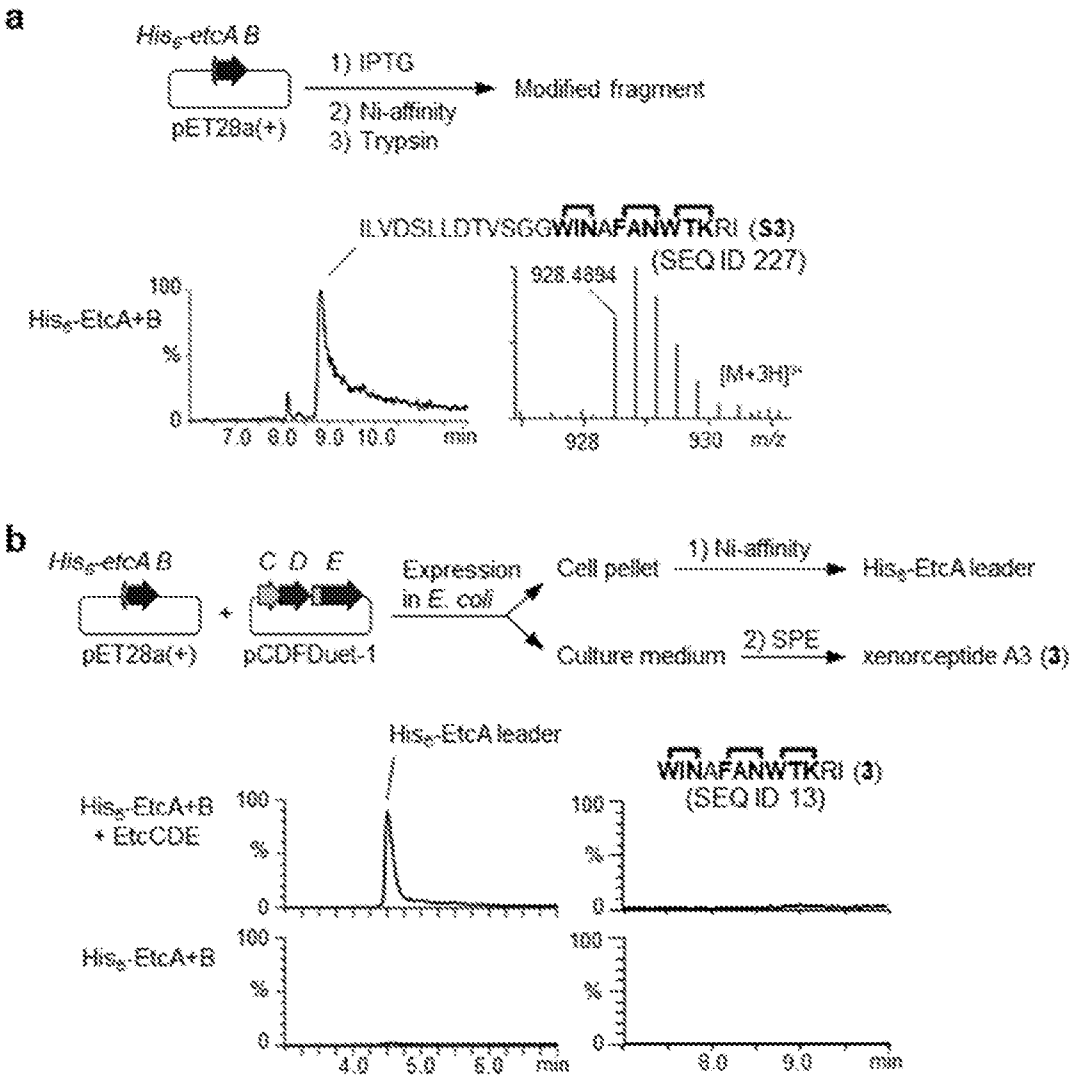

a
His6-etcA B
pET28a(+)
1) IPTG
2) Ni-affinity
3) Trypsin
Modified fragment
ILVDSLLDTVSGGWINAFANWTKRI (S3)
(SEQ ID 227)
928.4894
His6-EtcA+B
100
%
0
7.0 8.0 9.0 10.0 min
[M+3H]3+
928 930 m/z
b
His6-etcA B
C D E
pET28a(+)
pCDFDuet-1
Expression in E. coli
Cell pellet
1) Ni-affinity
His6-EtcA leader
Culture medium
2) SPE
xenorceptide A3 (3)
His6-EtcA leader
WINAFANWTKRI (3)
(SEQ ID 13)
His6-EtcA+B + EtcCDE
His6-EtcA+B
4.0 5.0 6.0 min
8.0 9.0 min

Figure 8

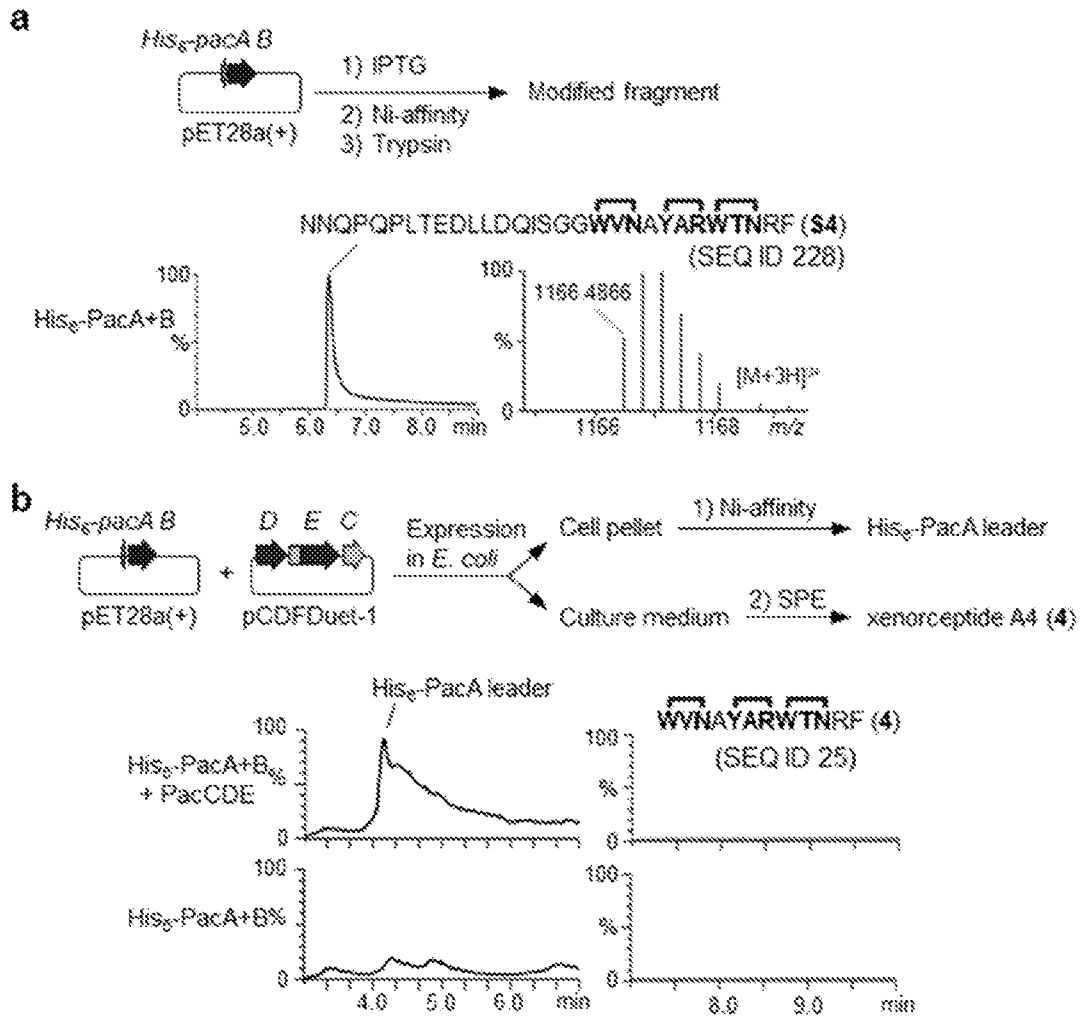

a
His6-pacA B
pET28a(+)
1) IPTG
2) Ni-affinity
3) Trypsin
Modified fragment
NNQPQPLTEDLLDQISGGWVNAYARWTNRF (S4)
(SEQ ID 228)
His6-PacA+B
1166.4866
[M+3H]3+
5.0 6.0 7.0 8.0 min
1168 1168 m/z
b
His6-pacA B
D E C
pET28a(+)
pCDFDuet-1
Expression in E. coli
Cell pellet
1) Ni-affinity
His6-PacA leader
Culture medium
2) SPE
xenorceptide A4 (4)
His6-PacA leader
WVNAYARWTNRF (4)
(SEQ ID 25)
His6-PacA+B + PacCDE
His6-PacA+B
4.0 5.0 6.0 min
8.0 9.0 min

Figure 9

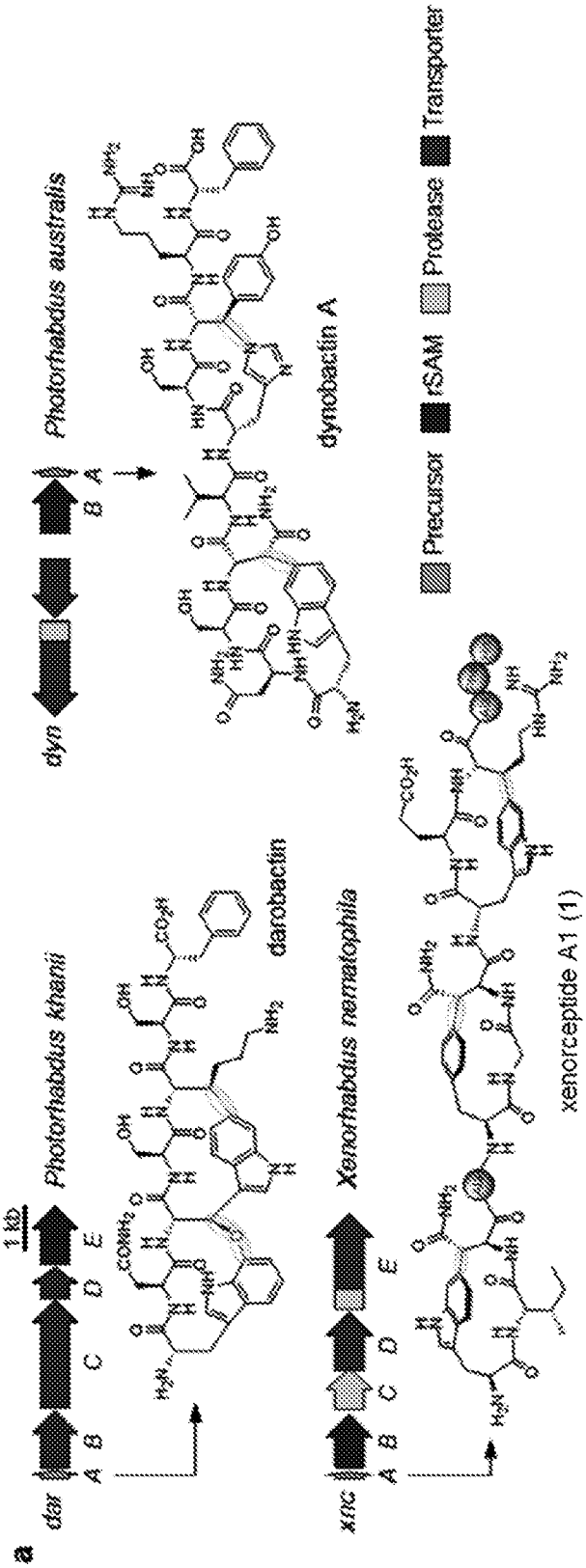

a
dar
A B C D E
1 kb
Photorhabdus khanii
darobactin
xnc
A B C D E
Xenorhabdus nematophila
xenorceptide A1 (1)
dyn
B A
Photorhabdus australis
dynobactin A
Precursor
rSAM
Protease
Transporter

Figure 10a

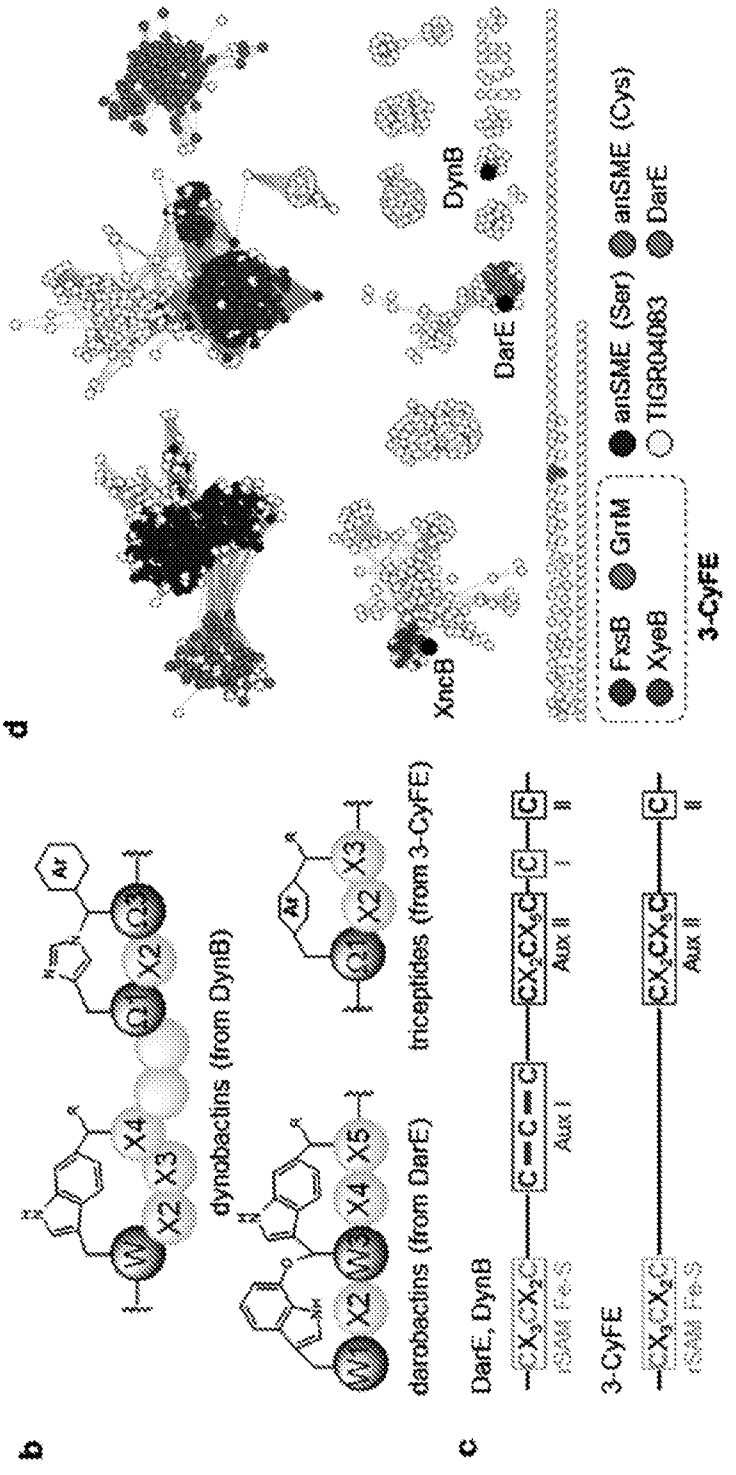

d
XncB
DarE
DynB
FxsB
GrmM
anSME (Ser)
anSME (Cys)
XyeB
TIGR04083
DarE
3-CyFE
b
dynobactins (from DynB)
darobactins (from DarE)
tricepticles (from 3-CyFE)
c
DarE, DynB
3-CyFE
Aux I
Aux II
SAM Fe-S

Figure 10b-d

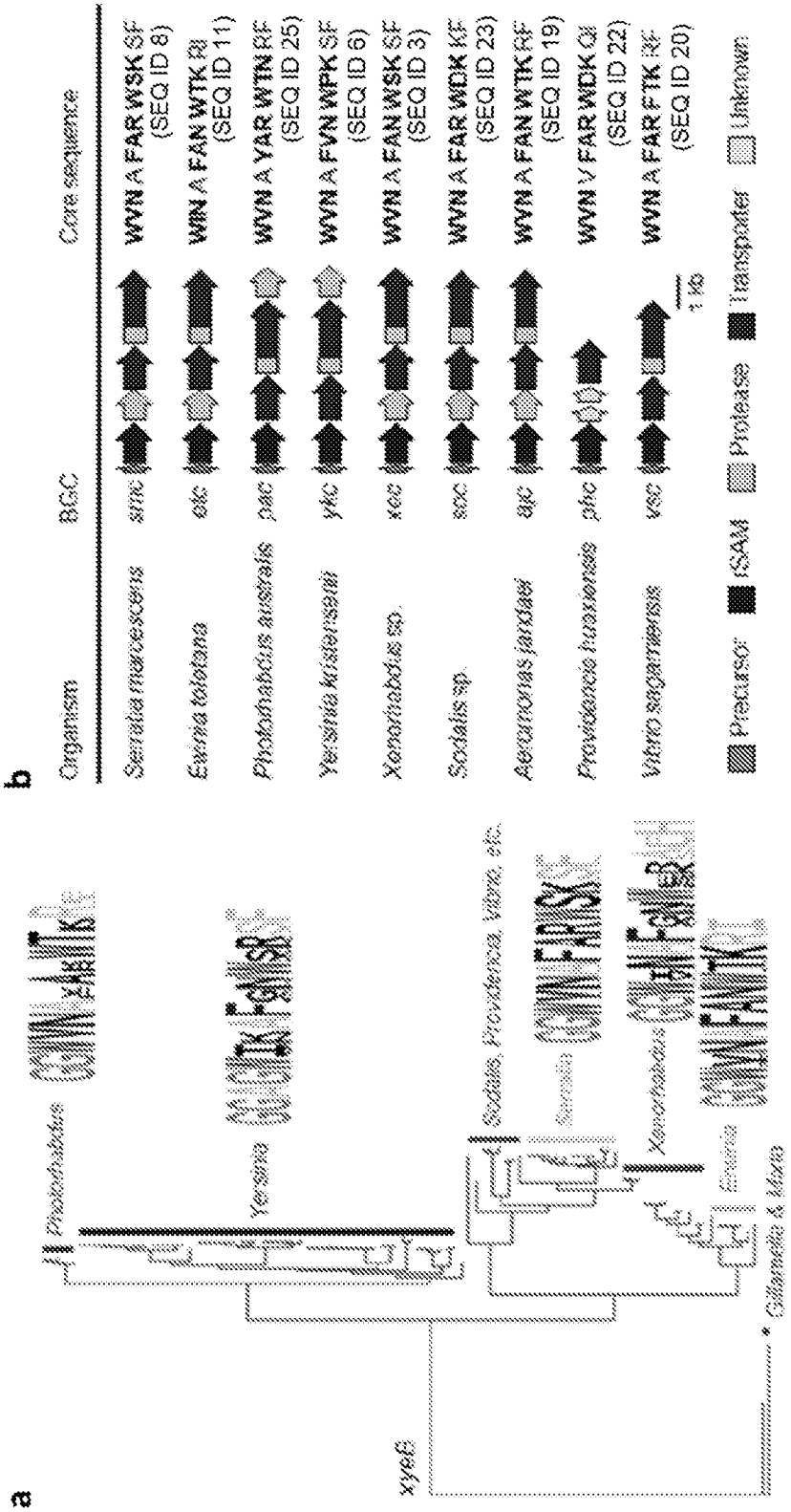

a
xyeB
Photorhabdus
Yersinia
Sodalis, Providencia, Vibrio, etc.
Serratia
Xenorhabdus
Enterobacter
Gilliamella & Mono
b
Organism
BGC
Core sequence
Serratia marcescens
sme
WVN A FAR WSK SF (SEQ ID 8)
Ewingella totoana
etc
WIN A FAN WTK Pl (SEQ ID 11)
Photorhabdus australis
pac
WVN A YAR WTN RF (SEQ ID 25)
Yersinia kristensenii
ykc
WVN A FVN WPK SF (SEQ ID 6)
Xenorhabdus sp.
xsc
WVN A FAN WSK SF (SEQ ID 3)
Sodalis sp.
soc
WVN A FAR WDK KF (SEQ ID 23)
Aeromonas jandaei
ajc
WVN A FAN WTK RF (SEQ ID 19)
Providencia huaxiensis
phc
WVN V FAR WDK Qt (SEQ ID 22)
Vibrio sagamiensis
vsc
WVN A FAR FTK RF (SEQ ID 20)
1 kb
Precursor
tSAM
Protease
Transporter
Unknown

Figure 11

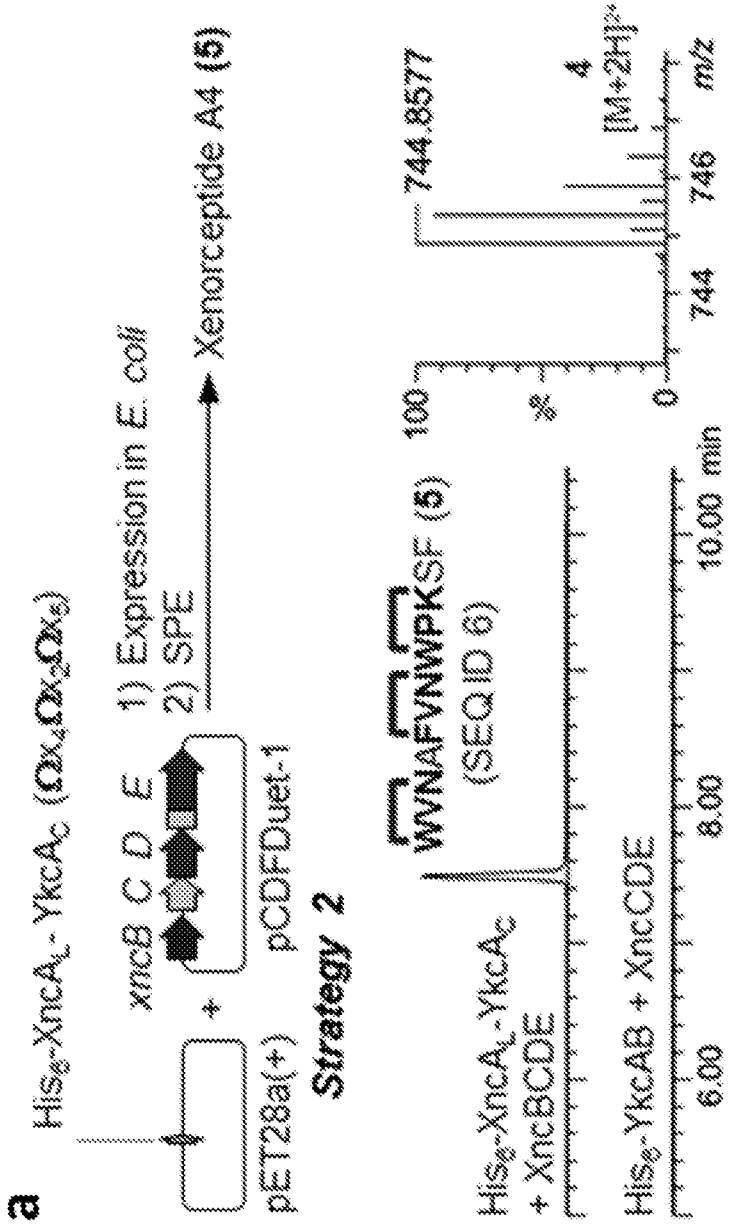

a
His6-XncAL- YkcAC (Ox1Ox2Ox3)
xncB C D E
1) Expression in E. coli
2) SPE
Xenorceptide A4 (5)
pET28a(+)
pCDFDuet-1
Strategy 2
WWNAFVNWPKSF (5)
(SEQ ID 6)
744.8577
4
[M+2H]2+
100
%
0
6.00
8.00
10.00 min
744
746
m/z
His6-XncAL-YkcAC
+ XncBCDE
His6-YkcAB + XncCDE

Figure 12a

b

Strain	BGC	Name	Sequence	SEQ ID	Strategy
Xenorhabdus nemotophila	*xnc*	Xenorceptide A1 (**1**)	**WIN** A **FGN WER** AFH	32	1
Serratia marcescens CAV1761	*smc*	Xenorceptide A2 (**2**)	**WVN** A **FAR WSK** SF	8	1
Ewinia toletana DAPP-PG 735	*etc*	Xenorceptide A3 (**3**)	**WIN** A **FAN WTK** RI	13	1
Photorhabdus australis DSM 17609	*pac*	Xenorceptide A4 (**4**)	**WVN** A **YAR WTN** RF	25	1
Yersinia kristensenii IP6945	*ykc*	Xenorceptide A5 (**5**)	**WVN** A **FVN WPK** SF	6	2
Xenorhabdus sp. BG5	*xec*	Xenorceptide A6 (**6**)	**WVN** A **FAN WSK** SF	3	2
Sodalis sp. dw_96	*soc*	Xenorceptide A7 (**7**)	**WVN** A **FAR WDK** KF	23	2
Aeromonas jandaei CN17A0119	*ajc*	Xenorceptide A8 (**8**)	**WVN** A **FAN WTK** RF	19	2
Providencia huaxiensis Pvs2	*phc*	Xenorceptide A9 (**9**)	**WVN** V **FAR WDK** QI	22	2 (trace)
Vibrio sagamiensis NBRC 104589	*vsc*	Xenorceptide A10 (**10**)	**WVN** A **FAR FTK** RF	20	2 (trace)

Figure 12b

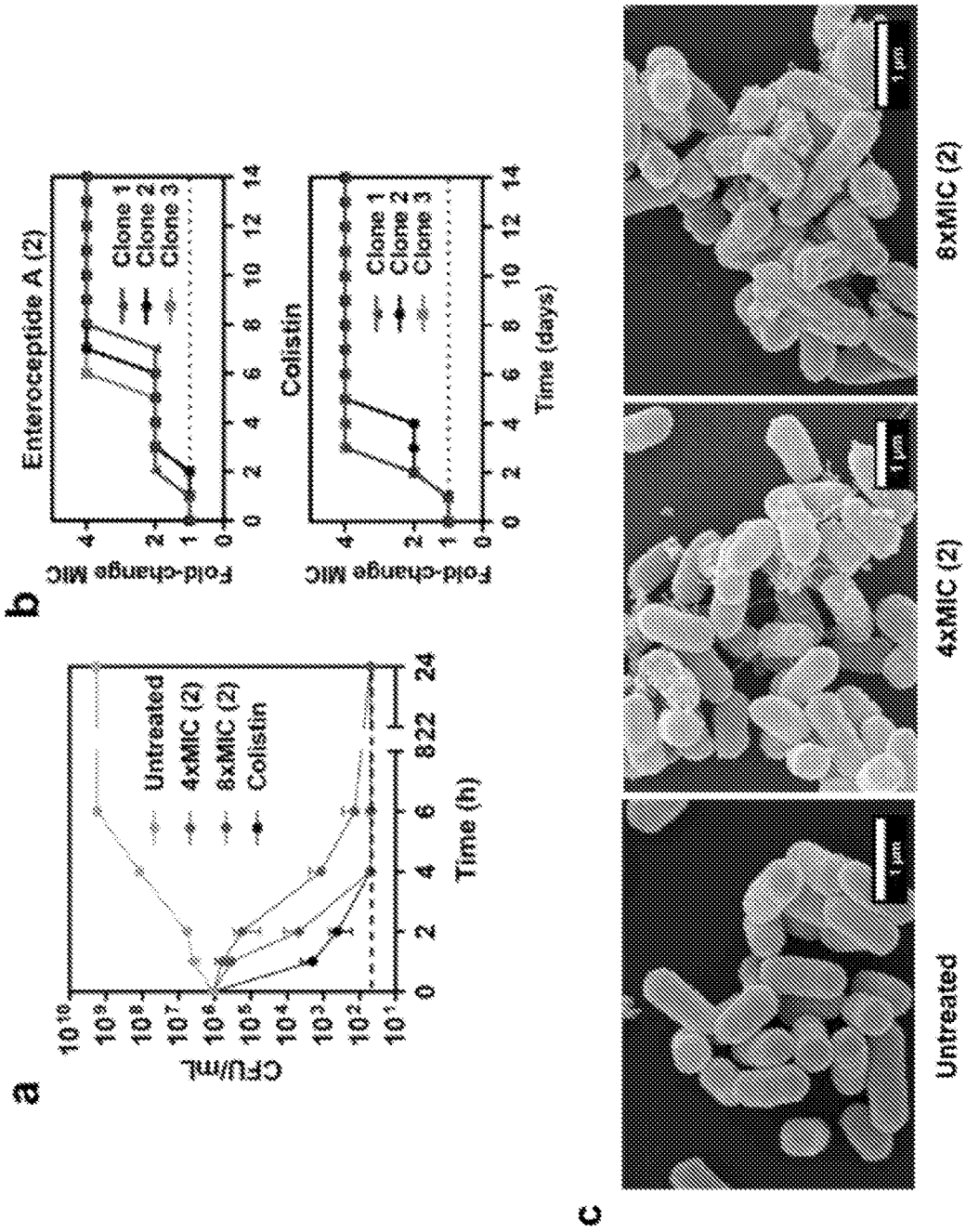

a
CFU/mL
Time (h)
Untreated
4xMIC (2)
8xMIC (2)
Colistin
b
Enteroceptide A (2)
Colistin
Fold-change MIC
Time (days)
Clone 1
Clone 2
Clone 3
c
Untreated
4xMIC (2)
8xMIC (2)
1 µm

Figure 13a-c

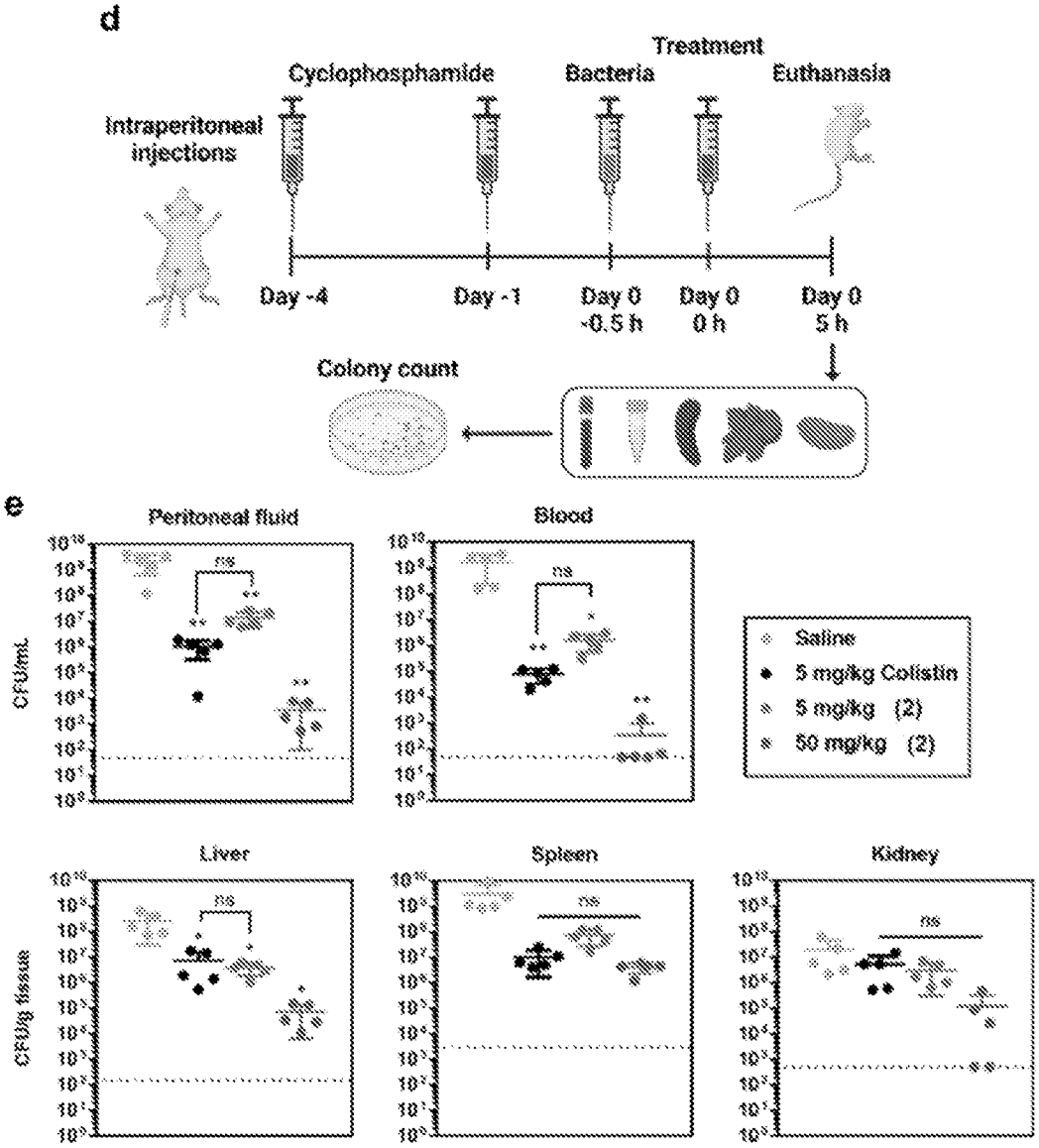

d
Treatment
Cyclophosphamide
Bacteria
Euthanasia
Intraperitoneal injections
Day -4
Day -1
Day 0 -0.5 h
Day 0 0 h
Day 0 5 h
Colony count
e
Peritoneal fluid
Blood
CFU/mL
ns
Saline
5 mg/kg Colistin
5 mg/kg (2)
50 mg/kg (2)
Liver
Spleen
Kidney
CFU/g tissue

Figure 13d-e

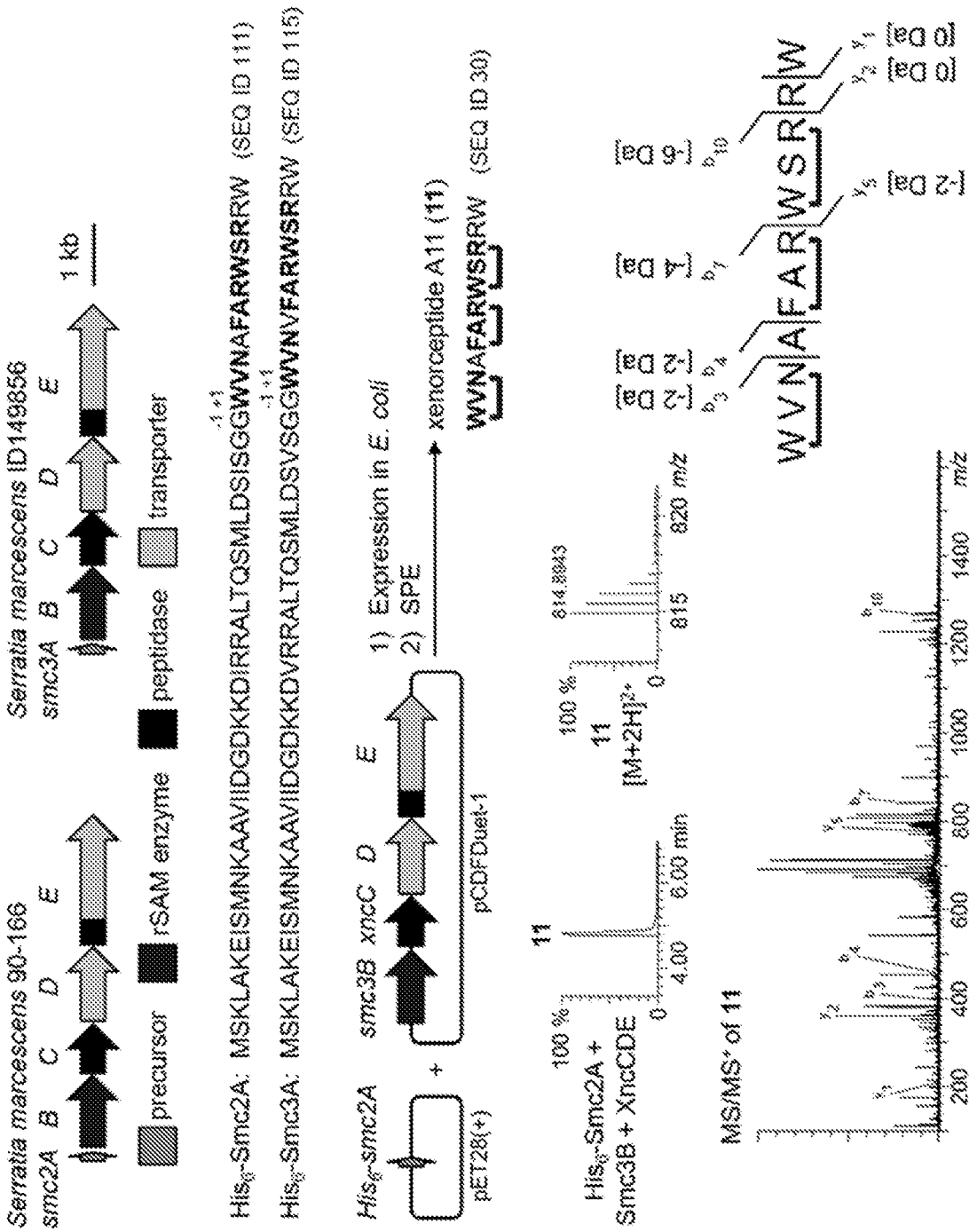

Serratia marcescens 90-166
smc2A B C D E
Serratia marcescens ID149856
smc3A B C D E
1 kb
precursor
rSAM enzyme
peptidase
transporter
His6-Smc2A: MSKLAKEISMNKAAVIIDGDKKDIRRALTQSMLDSISGGWVNAFARWSRRW (SEQ ID 111)
His6-Smc3A: MSKLAKEISMNKAAVIIDGDKKDVRRALTQSMLDSVSGGWVNVFARWSRRW (SEQ ID 115)
His6-smc2A
pET28(+)
smc3B xncC D E
pCDFDuet-1
1) Expression in E. coli
2) SPE
xenorceptide A11 (11)
WVNAFARWSRRW (SEQ ID 30)
11
100 %
0
4.00 8.00 min
His6-Smc2A + Smc3B + XncCDE
11
[M+2H]2+
814.8943
815 820 m/z
MS/MS* of 11
WVNAFARWSRRW
200 400 600 800 1000 1200 1400 m/z

Figure 14

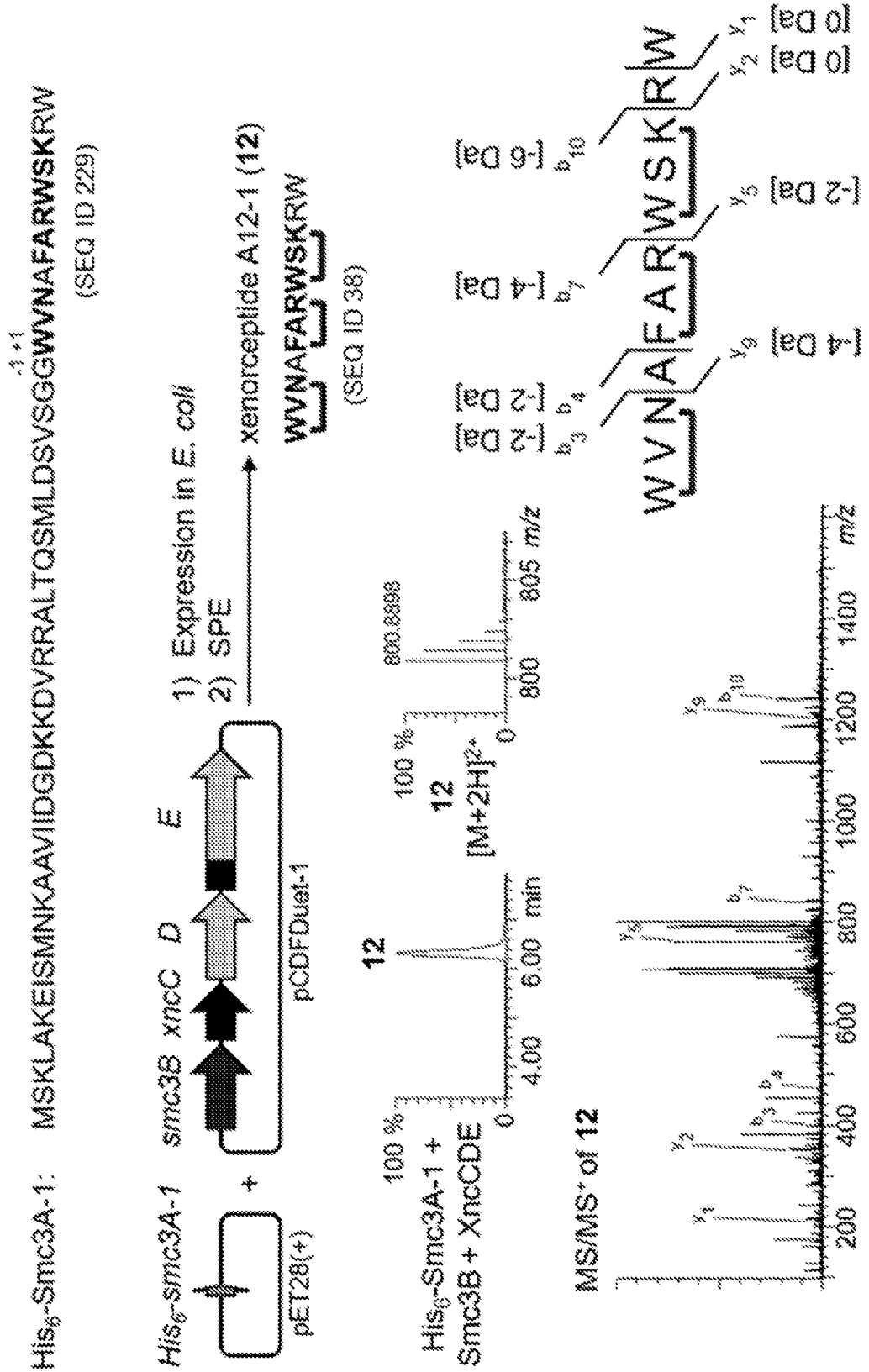

His6-Smc3A-1: MSKLAKEISMNKAAVIIDGDKKDVRRALTQSMLDSVSGGWVNAFARWSKRW
(SEQ ID 229)
His6-smc3A-1
pET28(+)
smc3B xncC D E
pCDFDuet-1
1) Expression in E. coli
2) SPE
xenorceptide A12-1 (12)
WVNAFARWSKRW
(SEQ ID 38)
12
His6-Smc3A-1 + Smc3B + XncCDE
12
[M+2H]2+
800.8898
MS/MS of 12
WVNAFARWSKRW
m/z
min

Figure 15

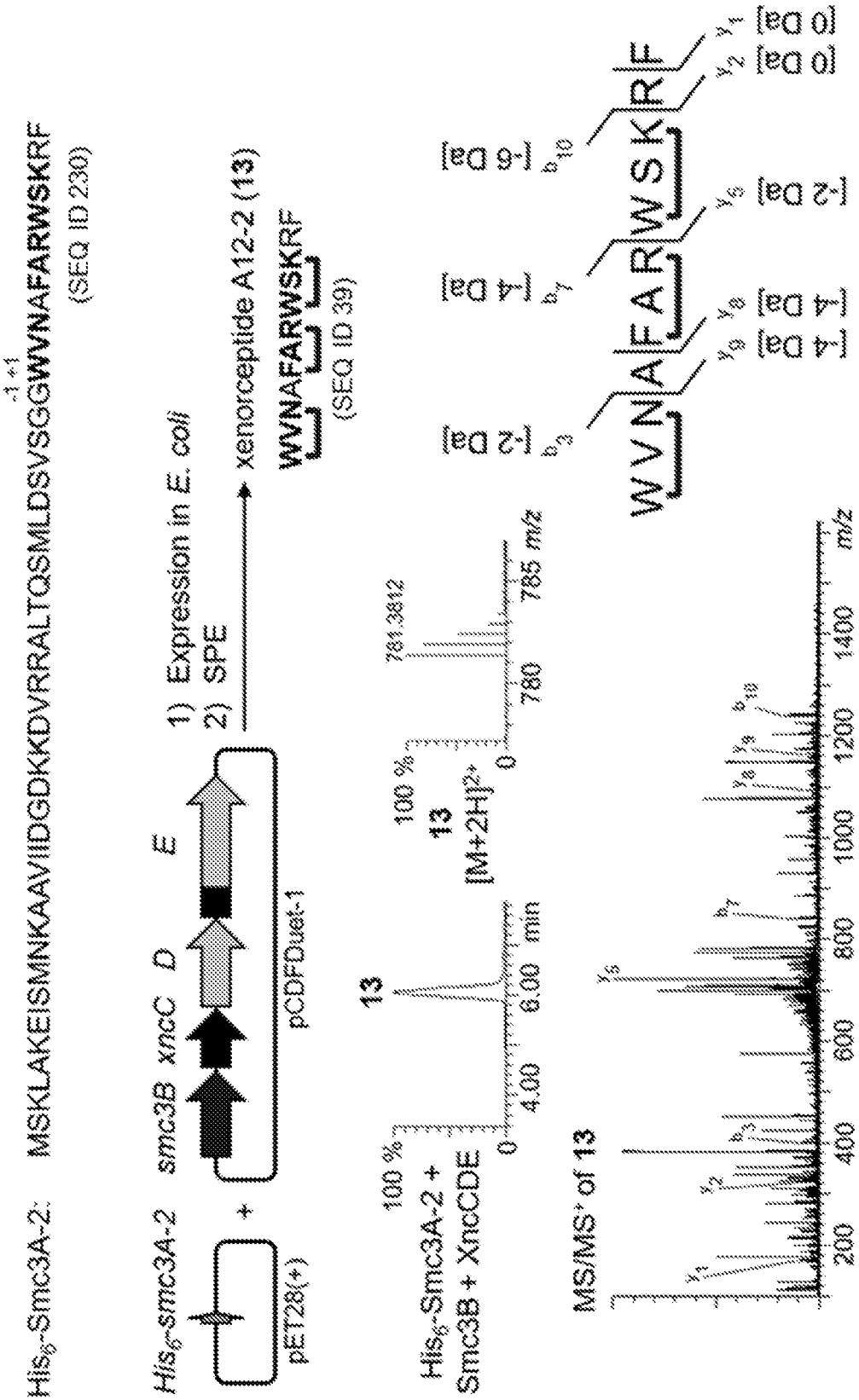

His6-Smc3A-2: MSKLAKEISMNKAAVIIDGDKKDVRRALTQSMLDSVSGGWVNAFARWSKRF
(SEQ ID 230)
-1 +1
His6-smc3A-2
smc3B xncC D E
pET28(+)
pCDFDuet-1
1) Expression in E. coli
2) SPE
xenorceptide A12-2 (13)
WVNAFARWSKRF
(SEQ ID 39)
His6-Smc3A-2 + Smc3B + XncCDE
13
100 %
0
4.00
6.00 min
13
[M+2H]2+
781.3812
780
785 m/z
MS/MS' of 13
200
400
600
800
1000
1200
1400 m/z
W V N A F A R W S K R F

Figure 16

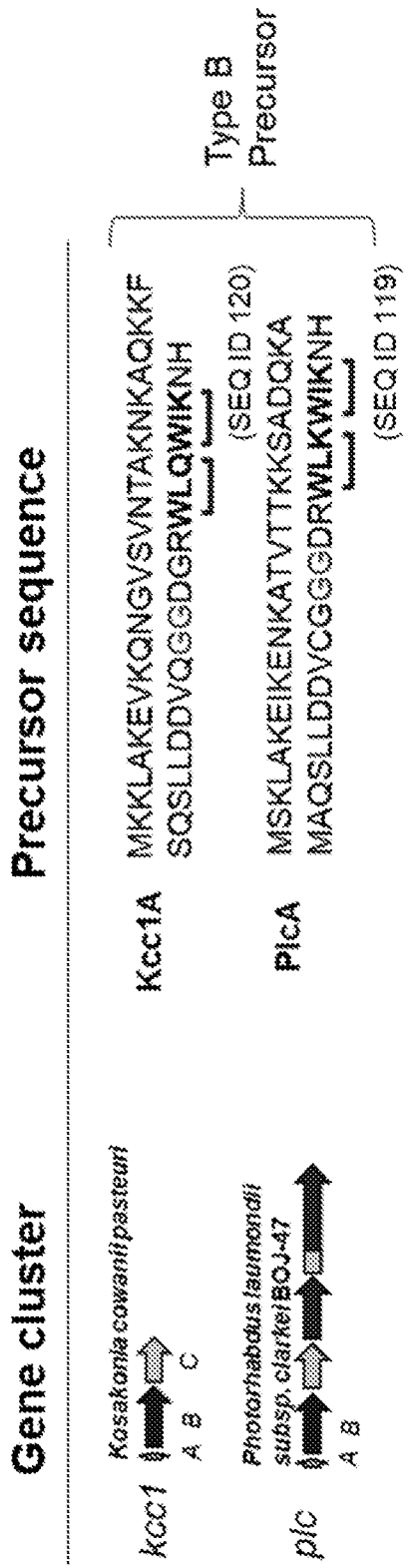


Figure 17 (cont.)

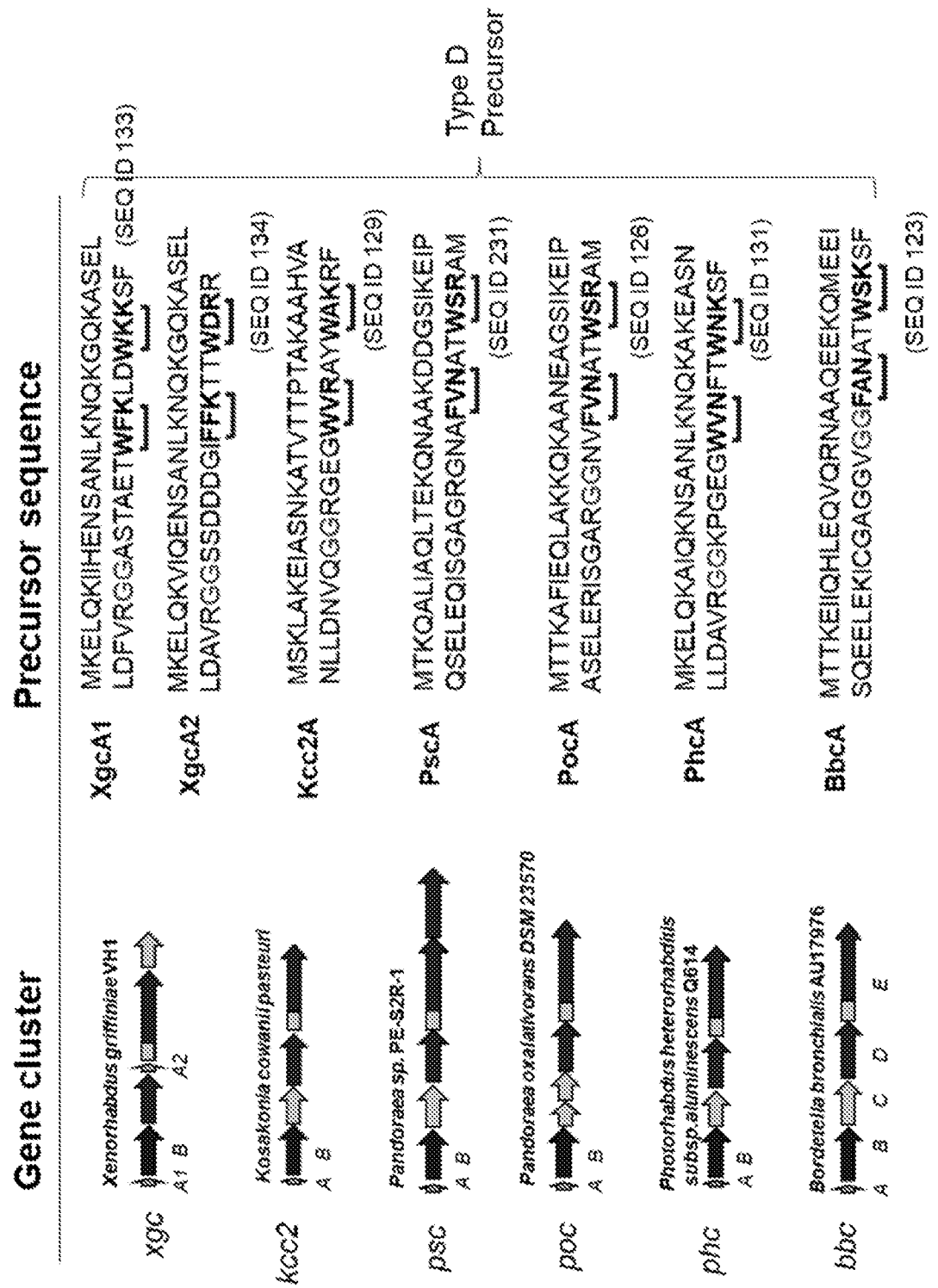


Figure 17 (end)

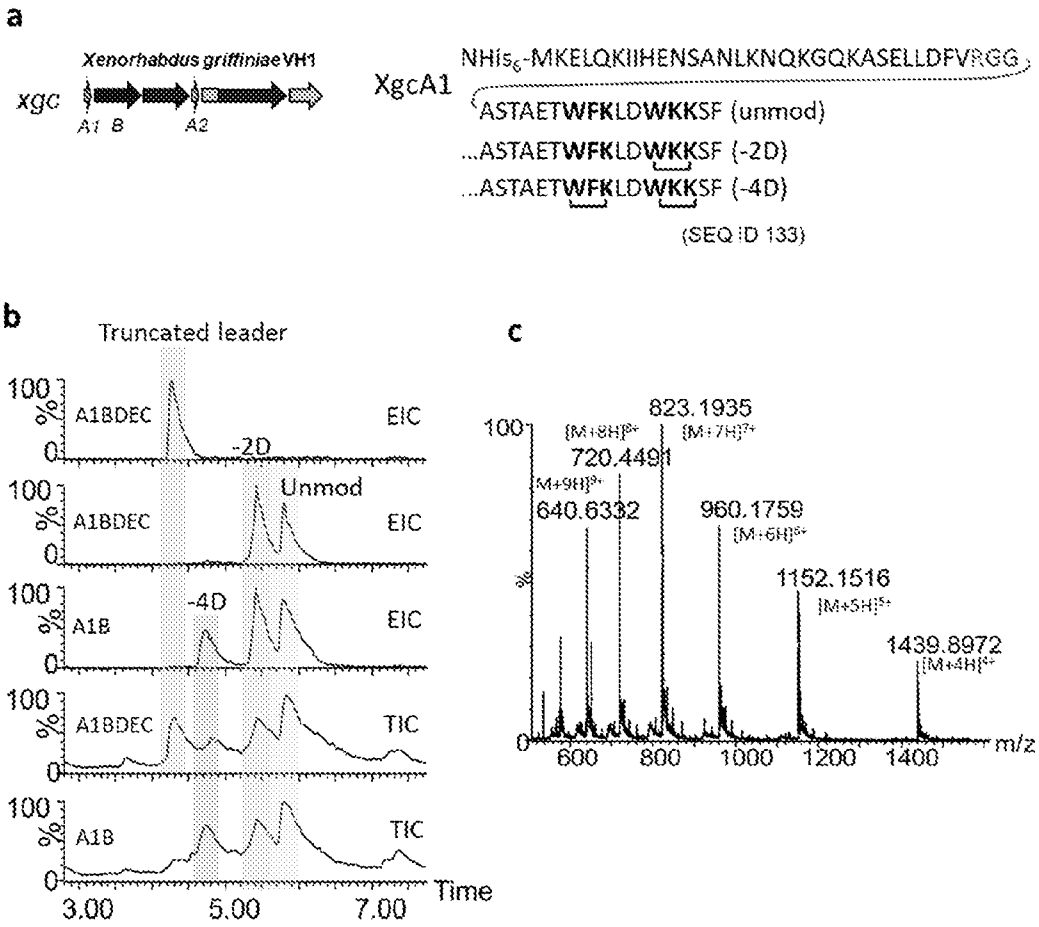

a
Xenorhabdus griffiniae VH1
xgc
A1 B
A2
XgcA1
NHis6-MKELQKIIHENSANLKNQKGQKASELLDFVRGG
ASTAETWFKLDWKKSF (unmod)
...ASTAETWFKLDWKKSF (-2D)
...ASTAETWFKLDWKKSF (-4D)
(SEQ ID 133)
b
Truncated leader
A1BDEC
EIC
-2D
Unmod
A1B
-4D
TIC
Time
3.00
5.00
7.00
c
823.1935
720.4491
640.6332
960.1759
1152.1516
1439.8972
m/z
600
800
1000
1200
1400

Figure 18

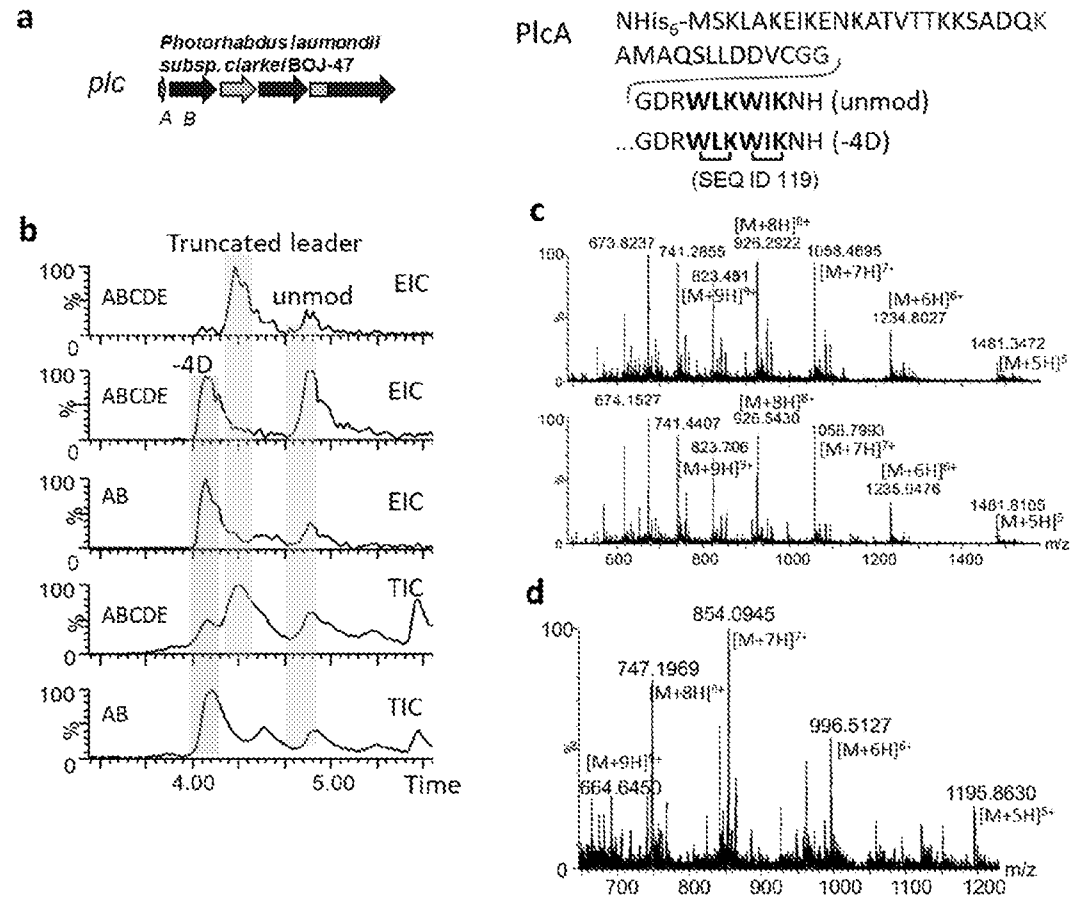
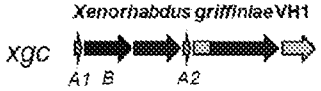


Figure 19



XgcA1 NHis$_6$-MKELQKIIHENSANLKNQKGQKASELLDFVGG
ASTAETWFKLDWKKSF (SEQ ID 133)

XgcA2 NHis$_6$-MKELQKVIQENSANLKNQKGQKASELLDAVRGG
SSDDDGIFFKTTWDRR (SEQ ID 134)

Figure 20

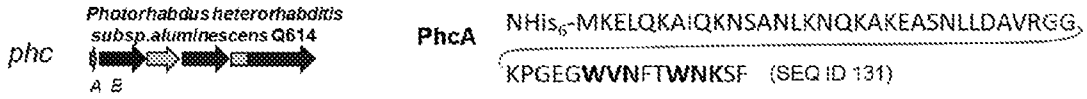
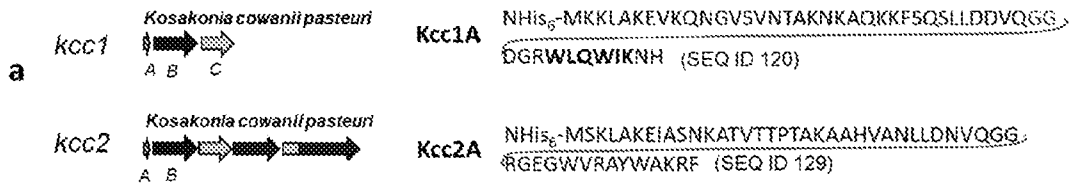
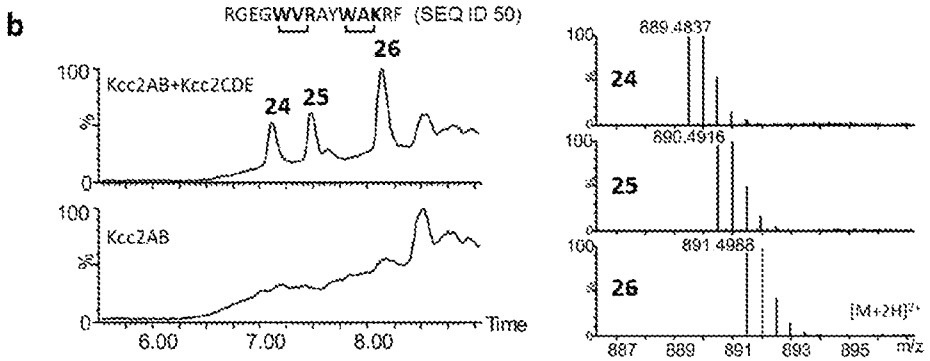


Figure 21





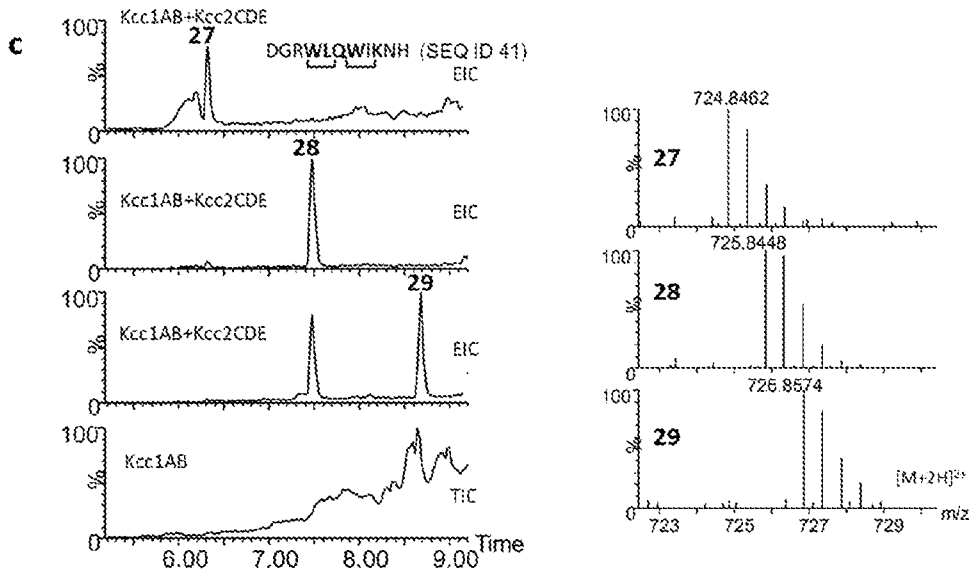


Figure 22

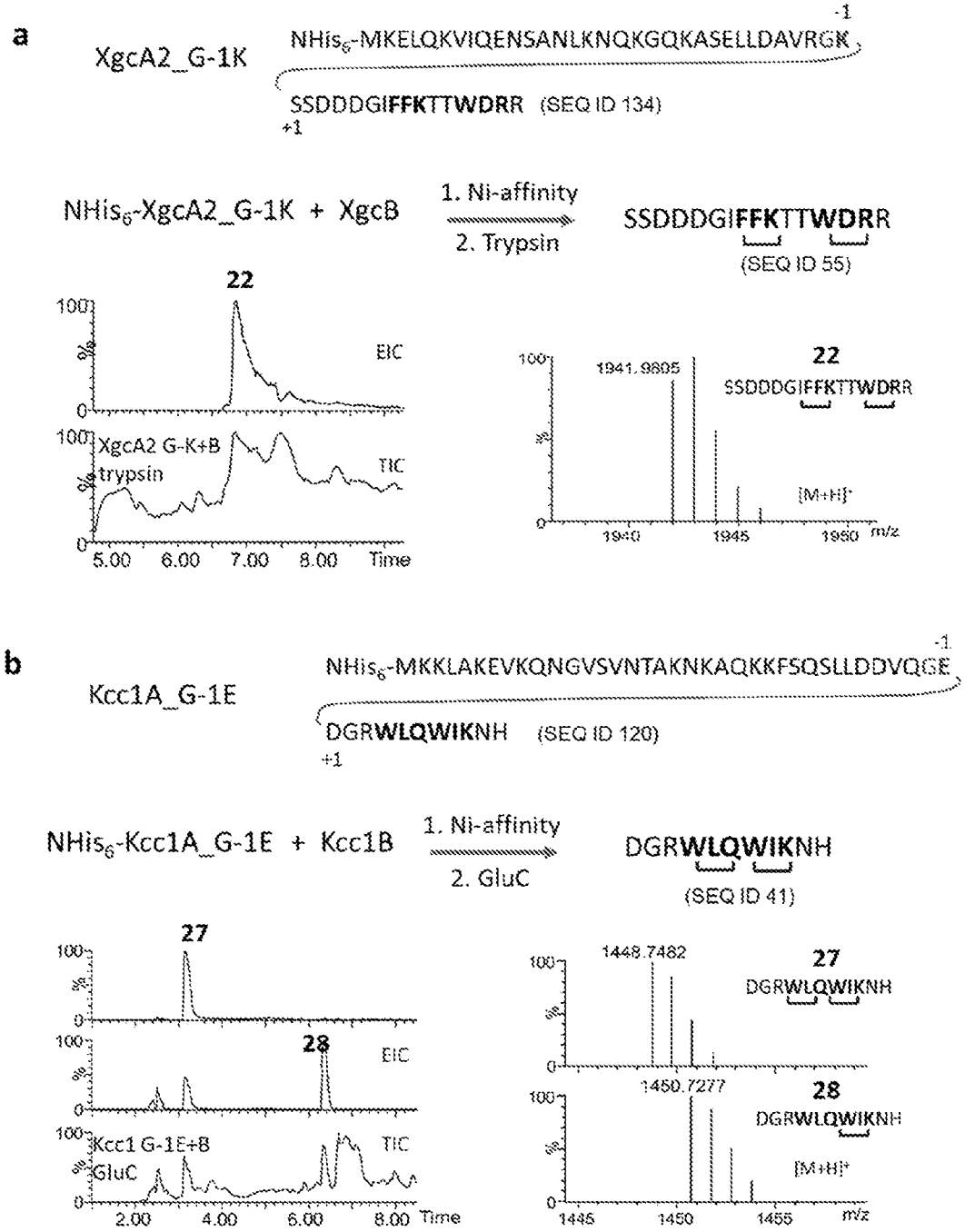

a
XgcA2_G-1K
NHis6-MKELQKVIQENSANLKNQKGQKASELLDAVRGK
-1
SSDDDGIFFKTTWDRR (SEQ ID 134)
+1
NHis6-XgcA2_G-1K + XgcB
1. Ni-affinity
2. Trypsin
SSDDDGIFFKTTWDRR
(SEQ ID 55)
22
EIC
XgcA2 G-K+B
trypsin
TIC
5.00 6.00 7.00 8.00 Time
1941.9805
22
SSDDDGIFFKTTWDRR
[M+H]+
1940 1945 1950 m/z
b
Kcc1A_G-1E
NHis6-MKKLAKEVKQNGVSVNTAKNKAQKKFSQSLLDDVQGE
-1
DGRWLQWIKNH (SEQ ID 120)
+1
NHis6-Kcc1A_G-1E + Kcc1B
1. Ni-affinity
2. GluC
DGRWLQWIKNH
(SEQ ID 41)
27
28
EIC
Kcc1 G-1E+B
GluC
TIC
2.00 4.00 6.00 8.00 Time
1448.7482
27
DGRWLQWIKNH
1450.7277
28
DGRWLQWIKNH
[M+H]+
1445 1450 1455 m/z

Figure 23a-b

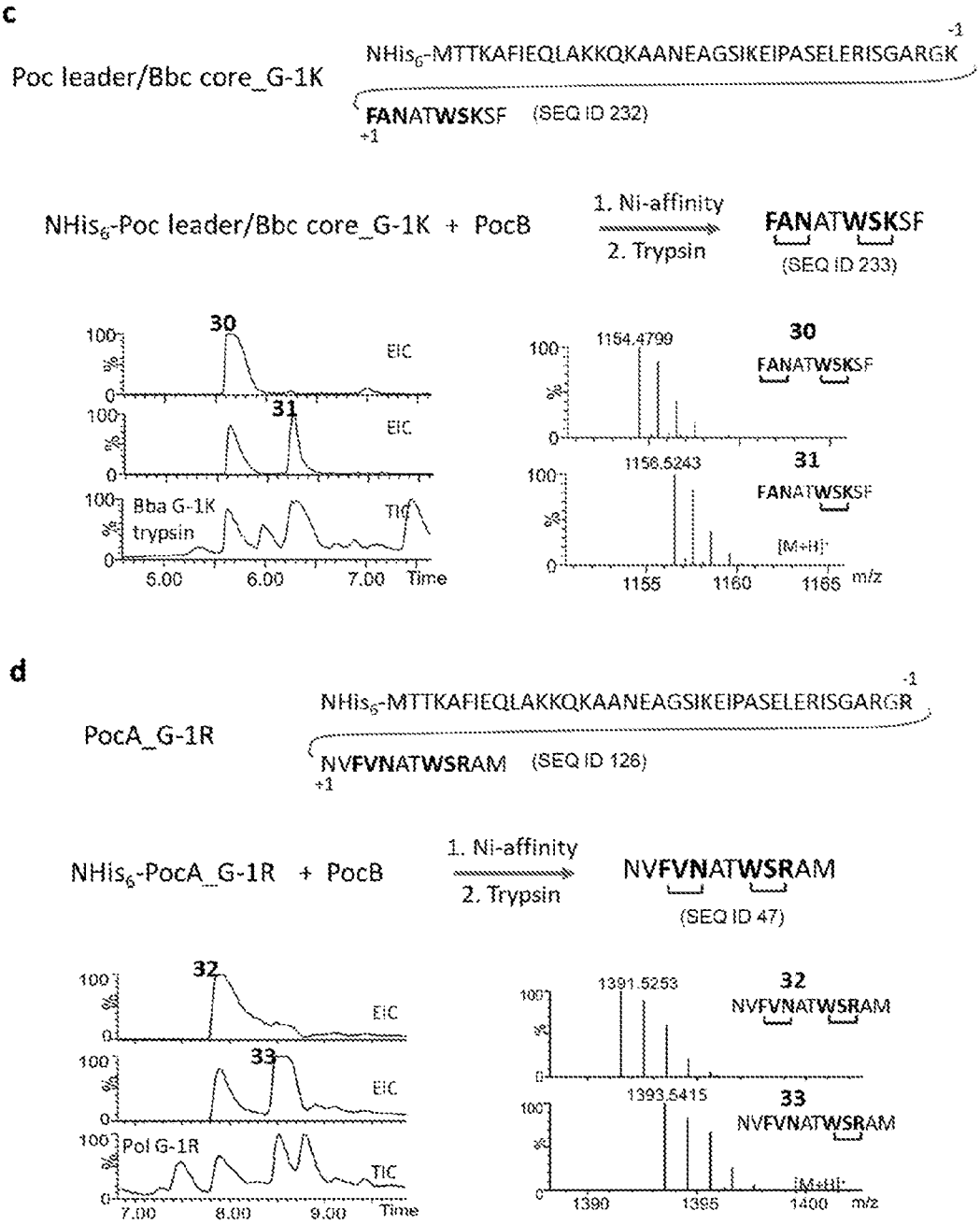

c
Poc leader/Bbc core_G-1K
NHis6-MTTKAFIEQLAKKQKAANEAGSIKEIPASELERISGARGK
-1
FANATWSKSF (SEQ ID 232)
+1
NHis6-Poc leader/Bbc core_G-1K + PocB
1. Ni-affinity
2. Trypsin
FANATWSKSF
(SEQ ID 233)
30
31
EIC
EIC
Bba G-1K trypsin
TIC
5.00
6.00
7.00
Time
1154.4799
30
FANATWSKSF
1156.5243
31
FANATWSKSF
[M+H]+
1155
1160
1165
m/z
d
PocA_G-1R
NHis6-MTTKAFIEQLAKKQKAANEAGSIKEIPASELERISGARGR
-1
NVFVNATWSRAM (SEQ ID 126)
+1
NHis6-PocA_G-1R + PocB
1. Ni-affinity
2. Trypsin
NVFVNATWSRAM
(SEQ ID 47)
32
33
EIC
EIC
Pol G-1R
TIC
7.00
8.00
9.00
Time
1391.5253
32
NVFVNATWSRAM
1393.5415
33
NVFVNATWSRAM
[M+H]+
1390
1395
1400
m/z

Figure 23c-d

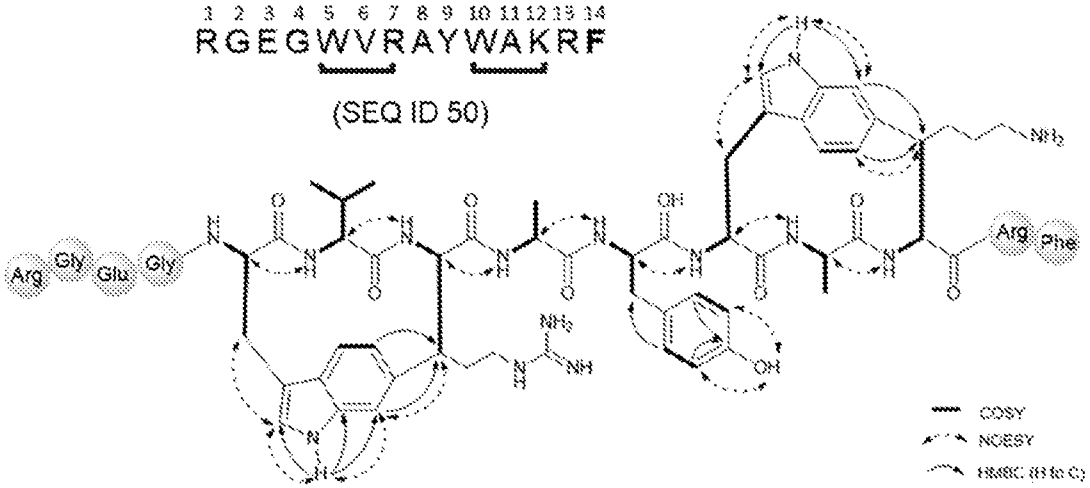


Figure 24

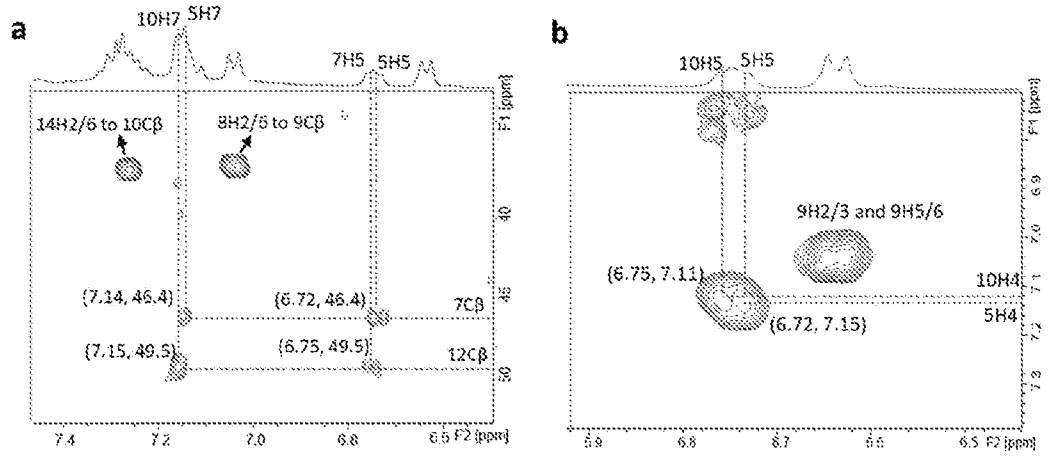


Figure 25

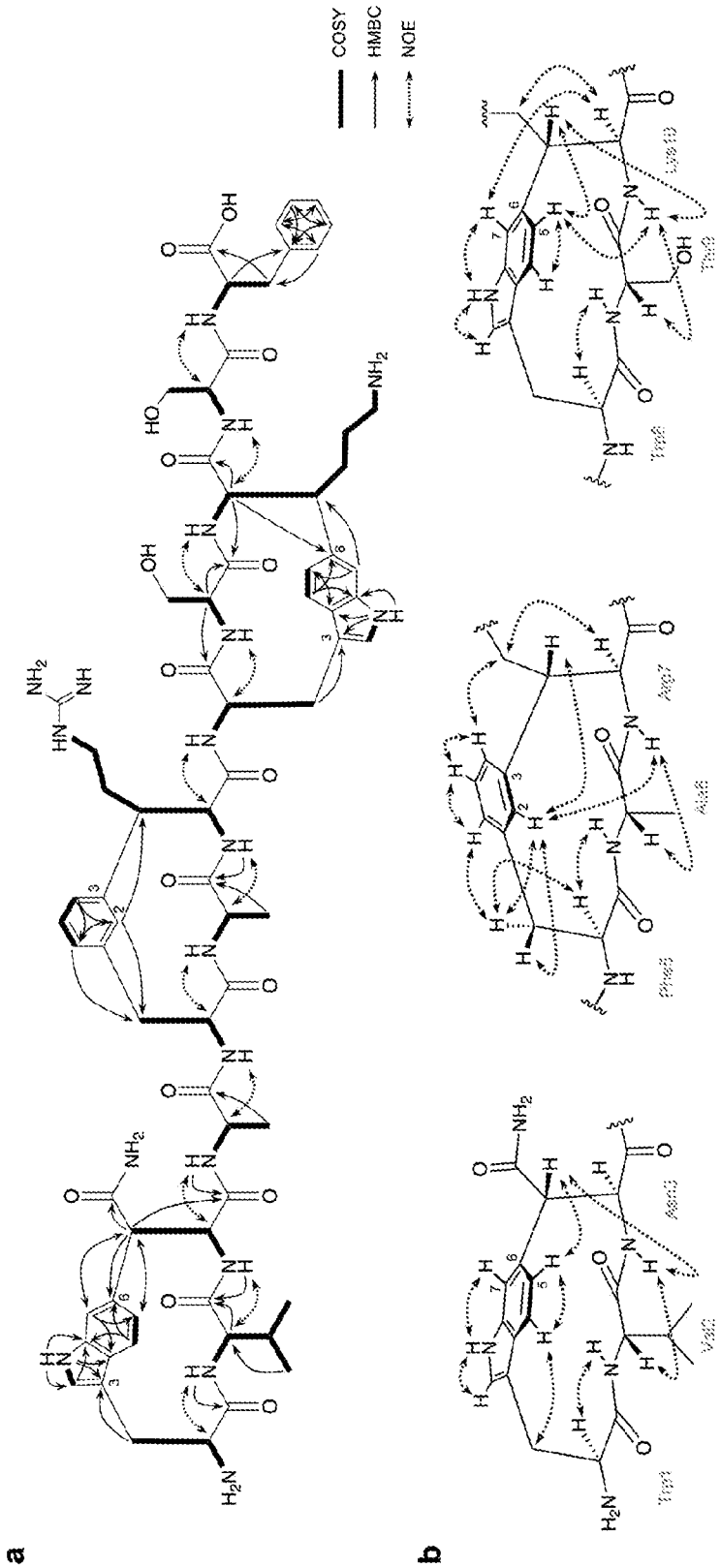


Figure 26

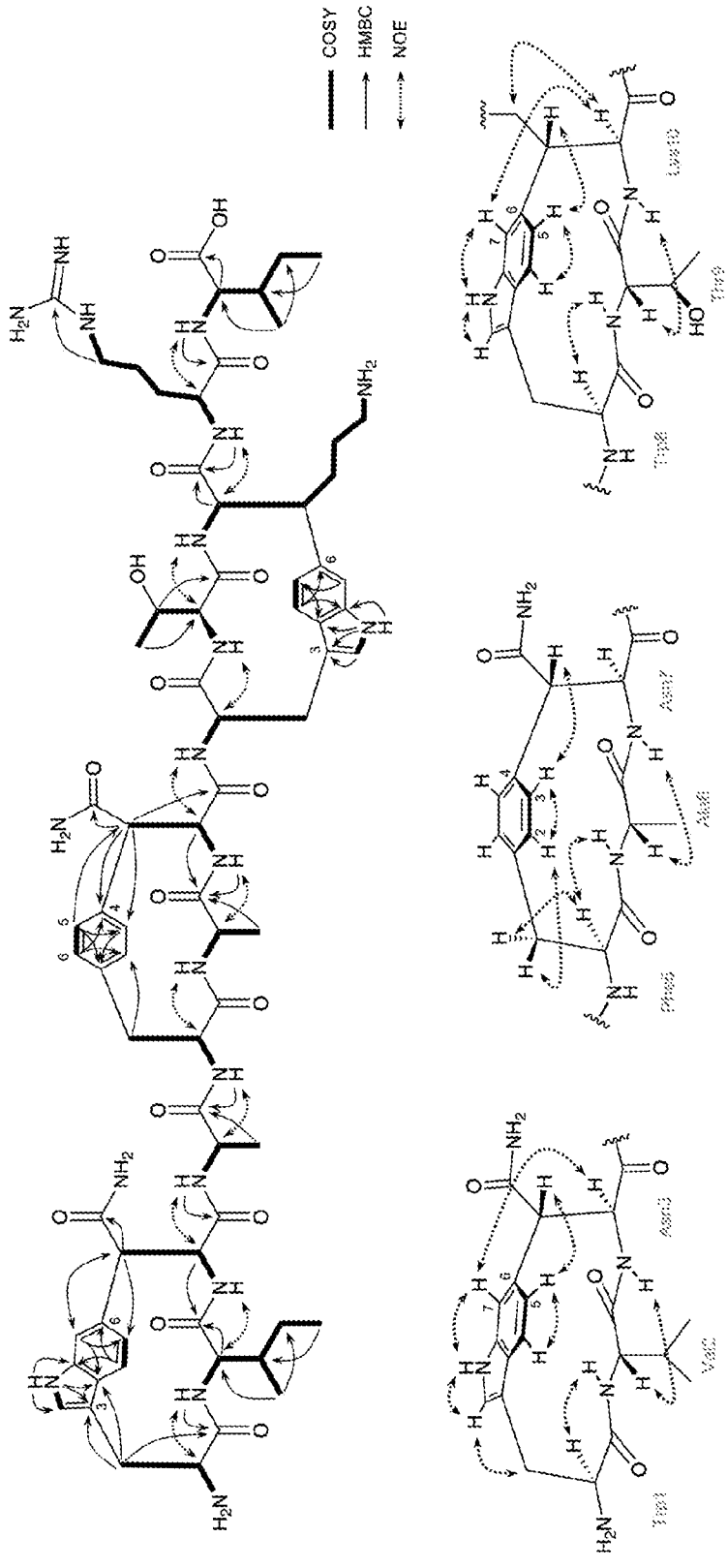


Figure 27

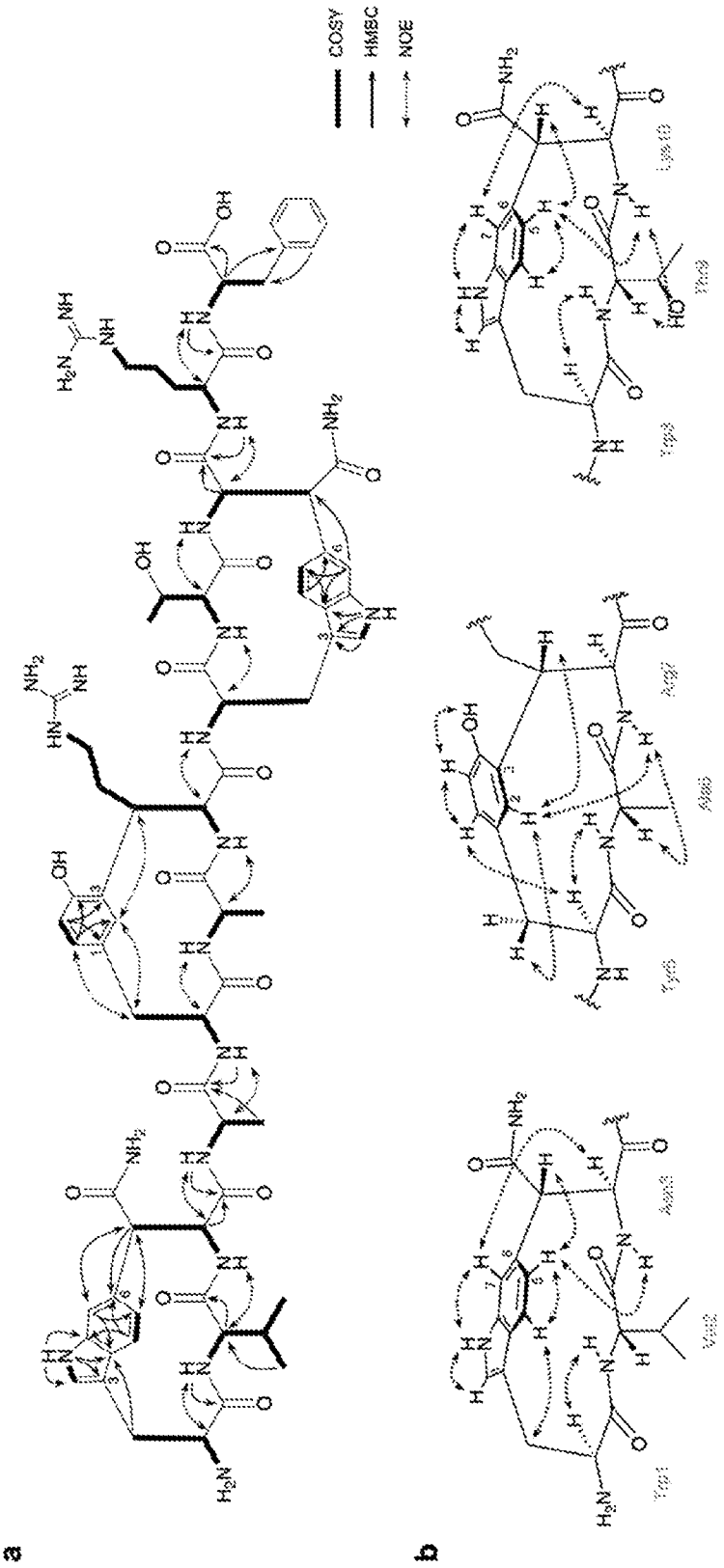


Figure 28

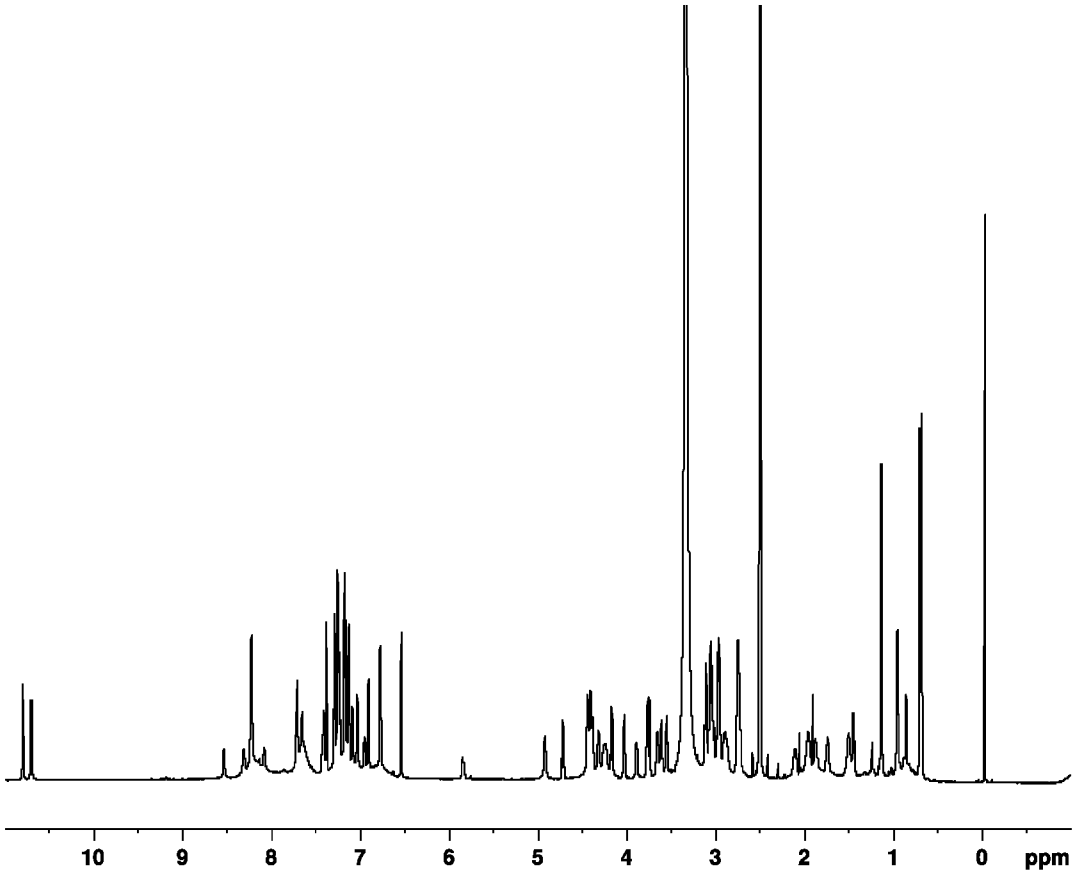


Figure 29

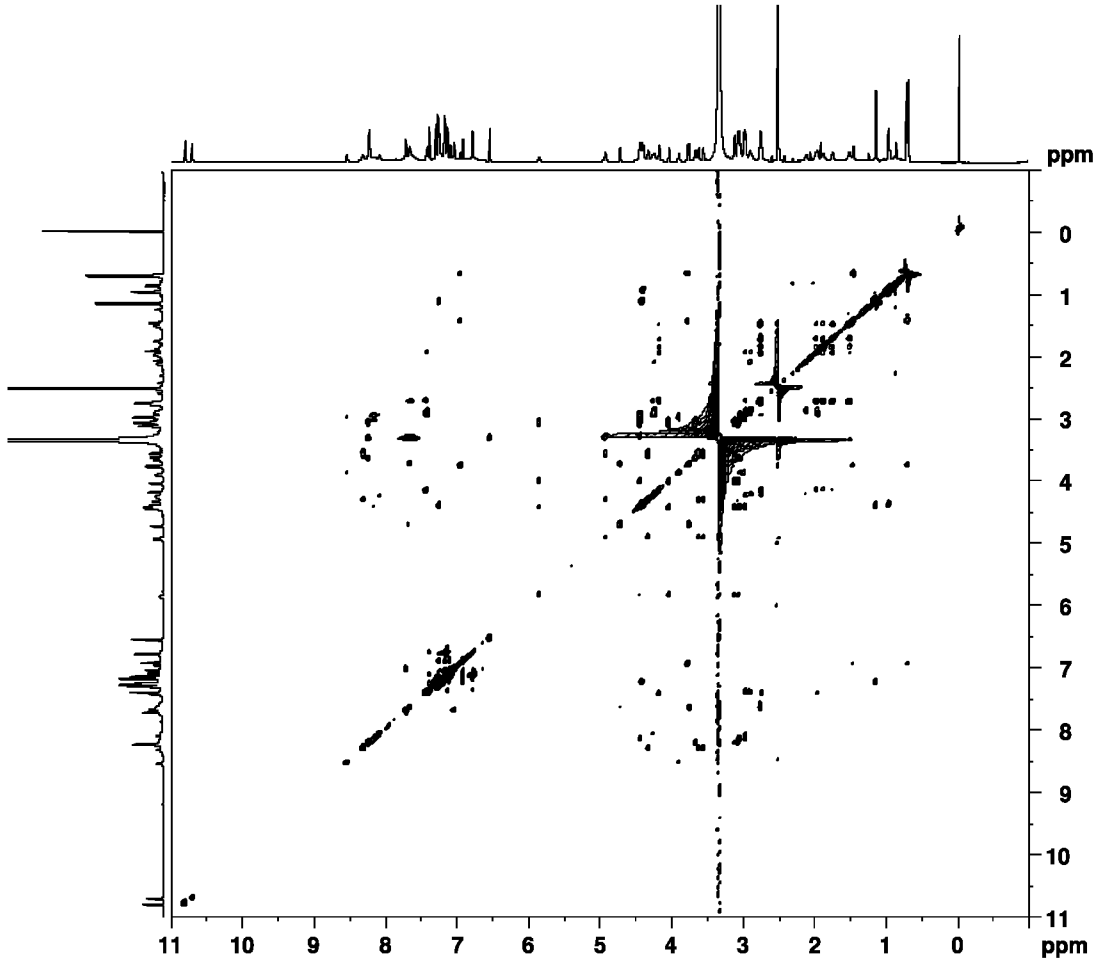


Figure 30

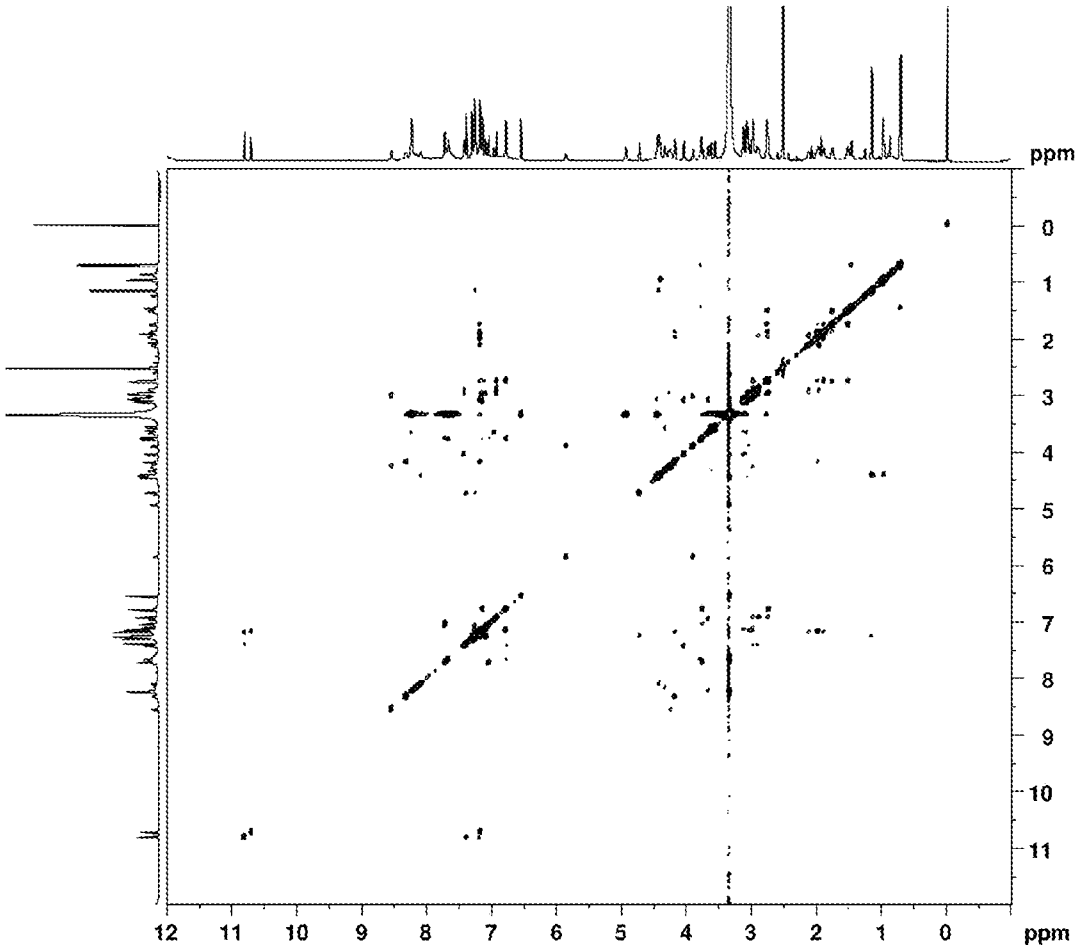


Figure 31

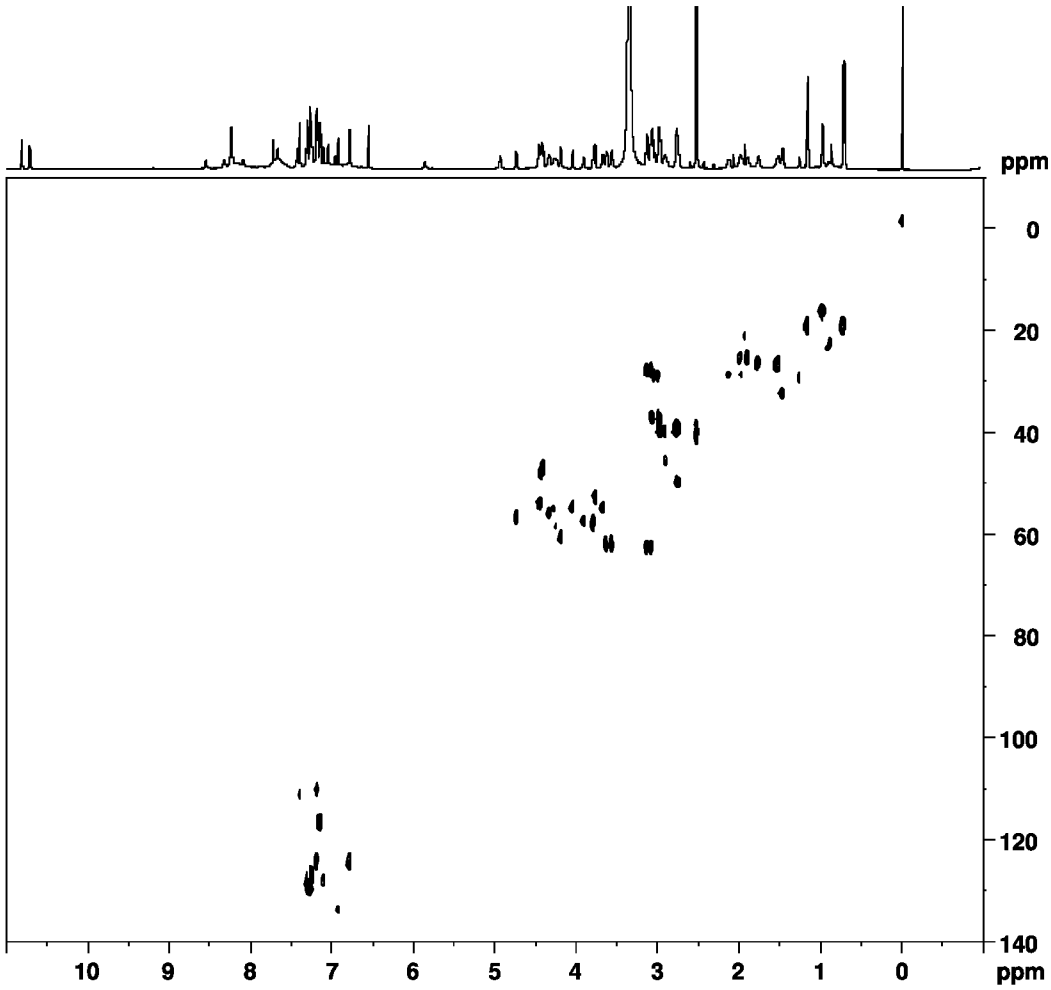


Figure 32

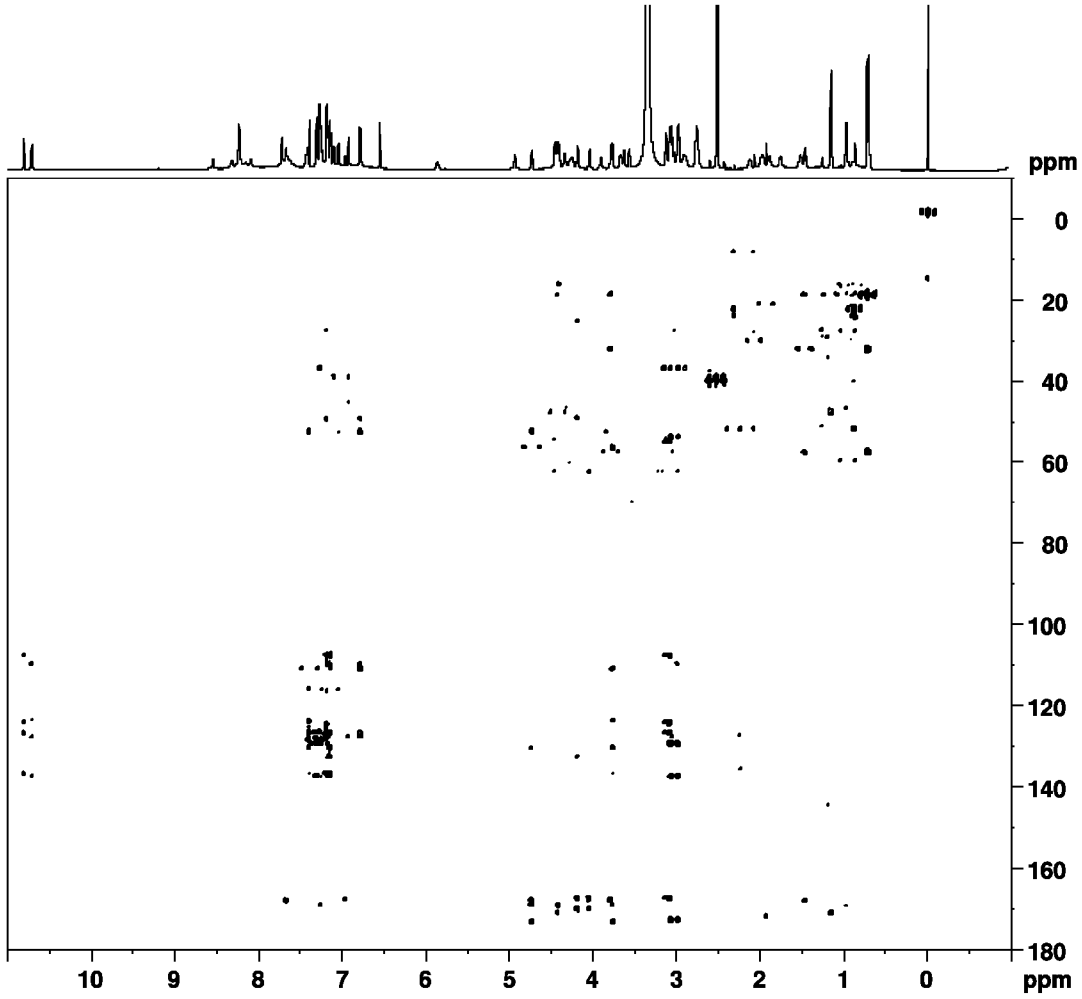


Figure 33

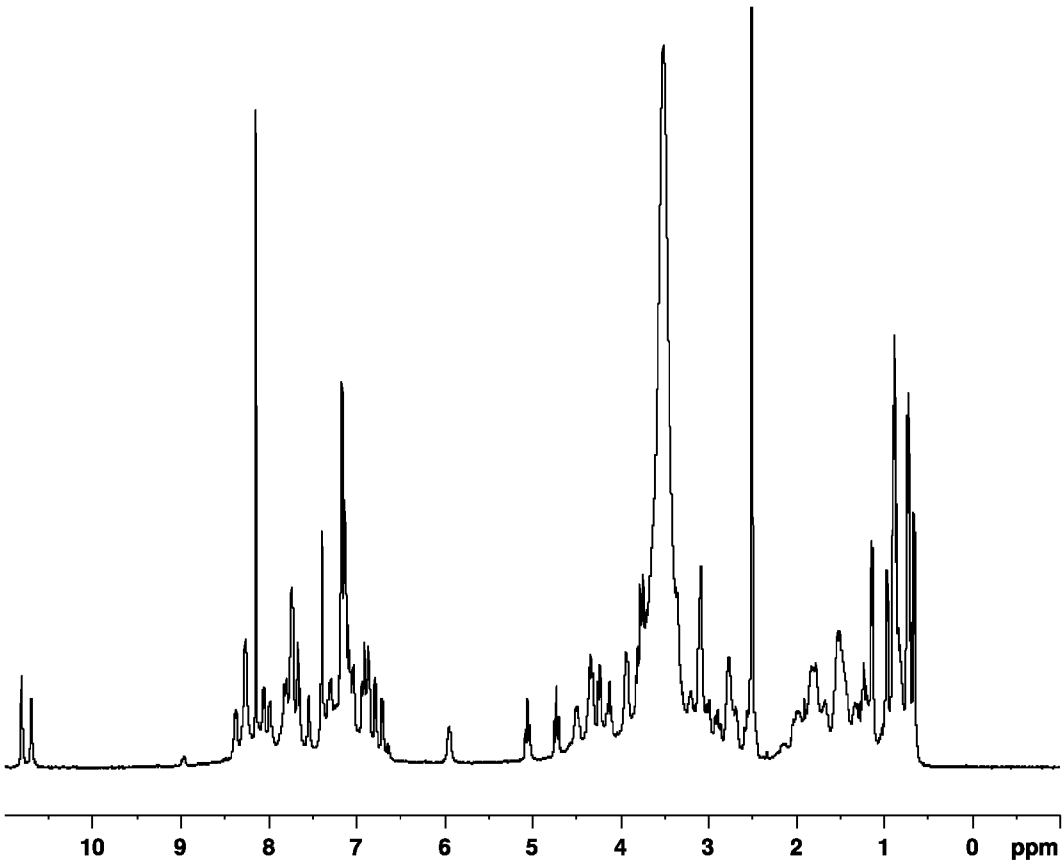


Figure 34

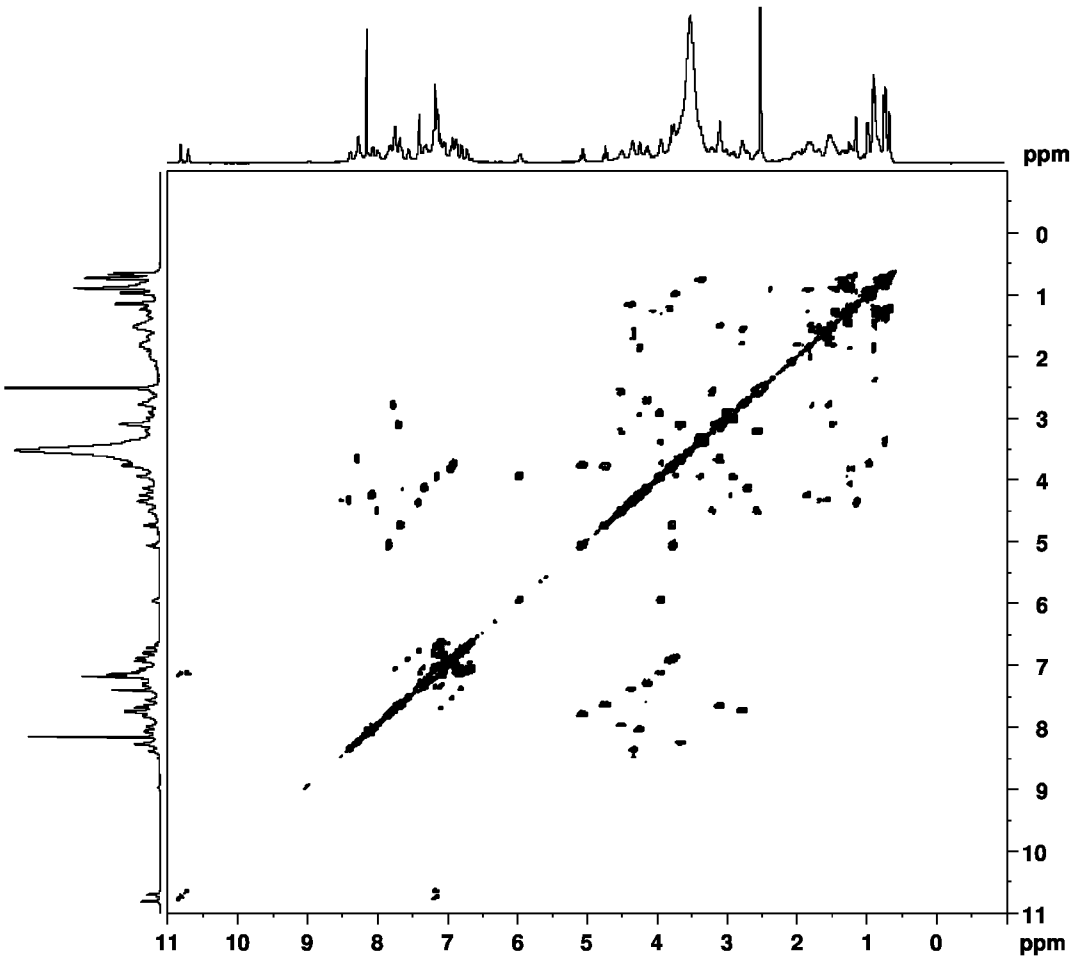


Figure 35

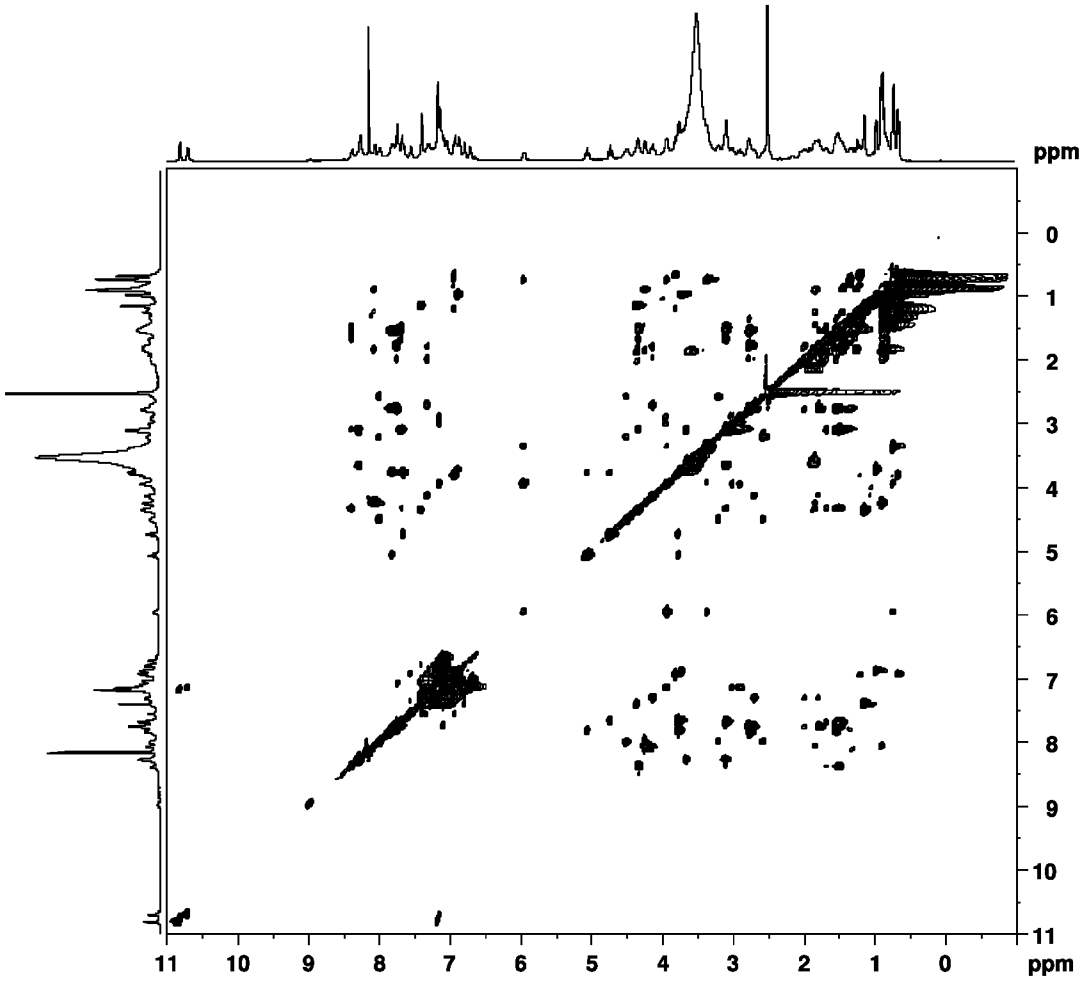


Figure 36

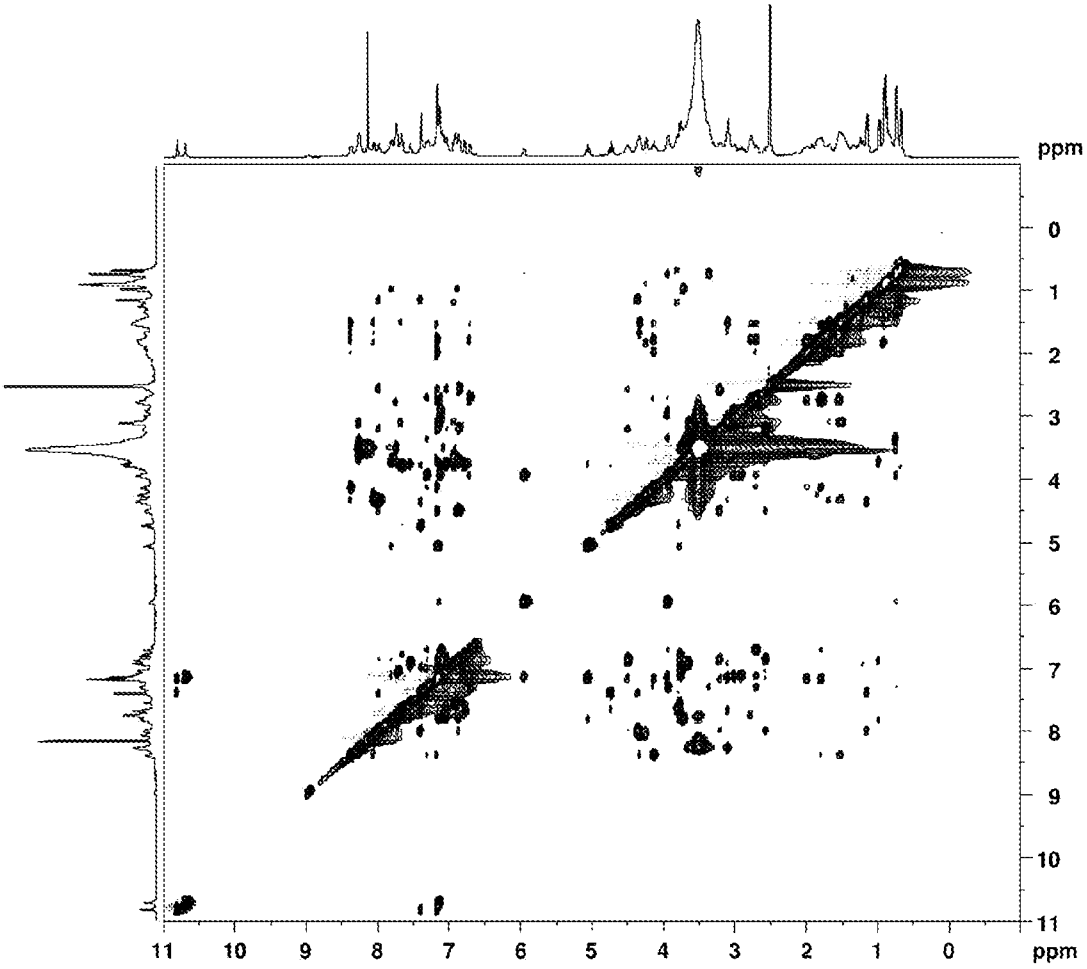


Figure 37

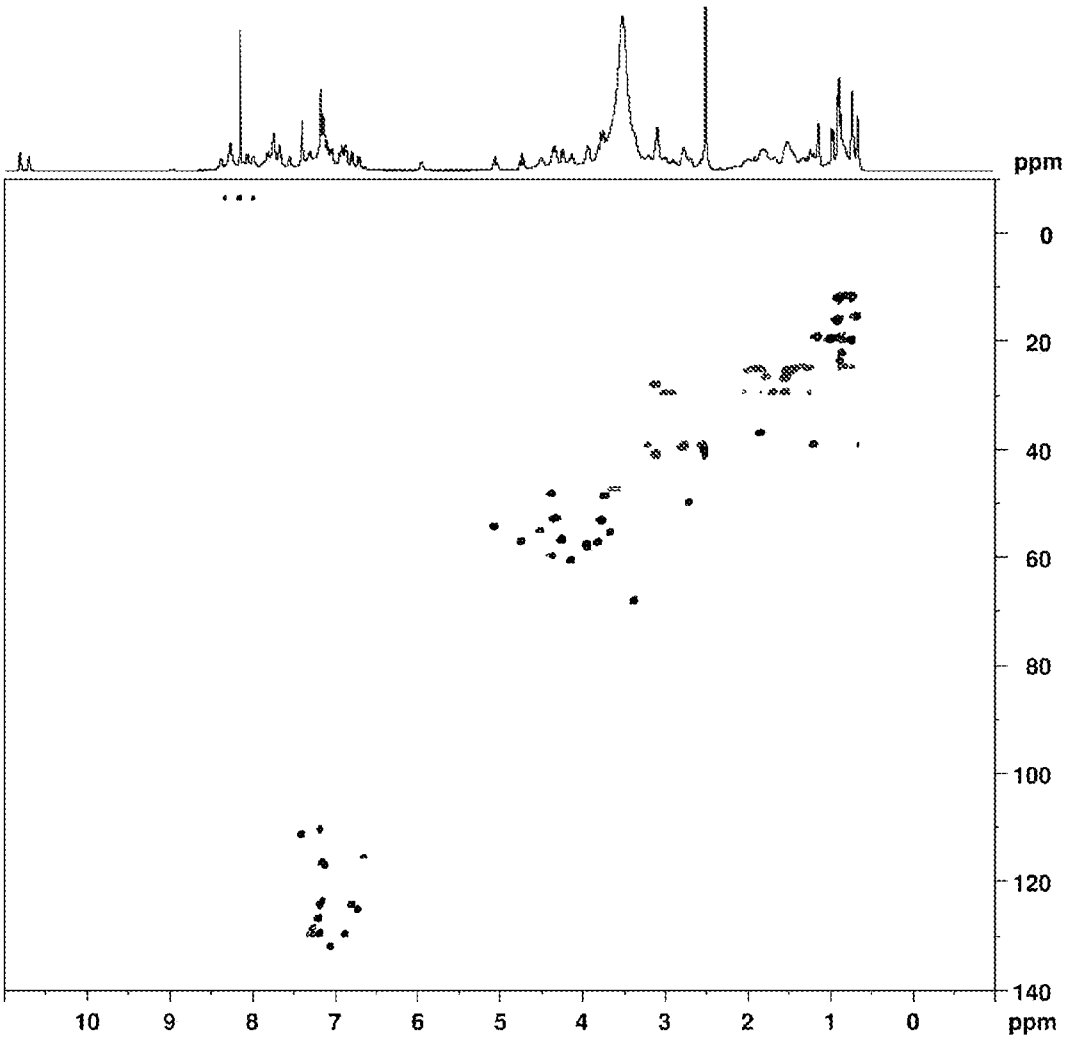


Figure 38

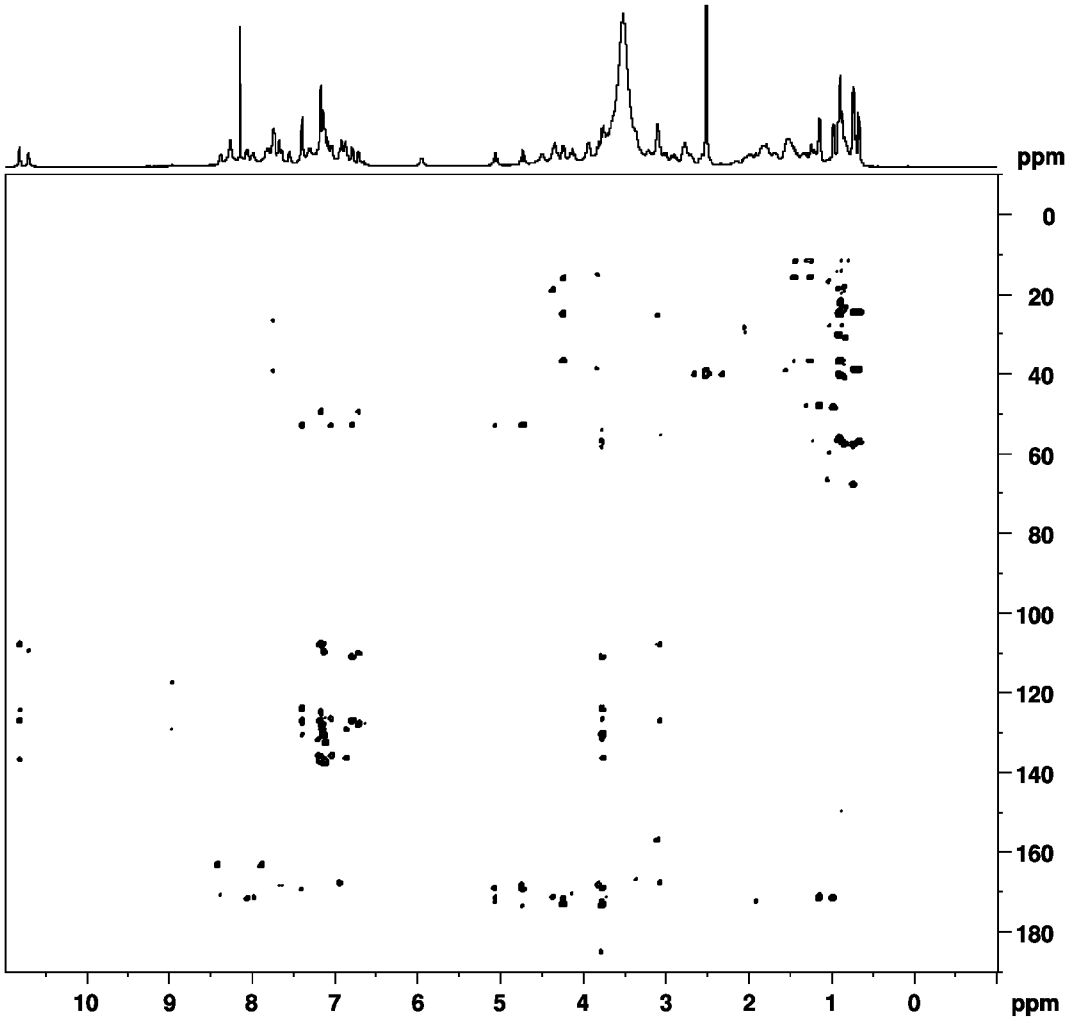


Figure 39

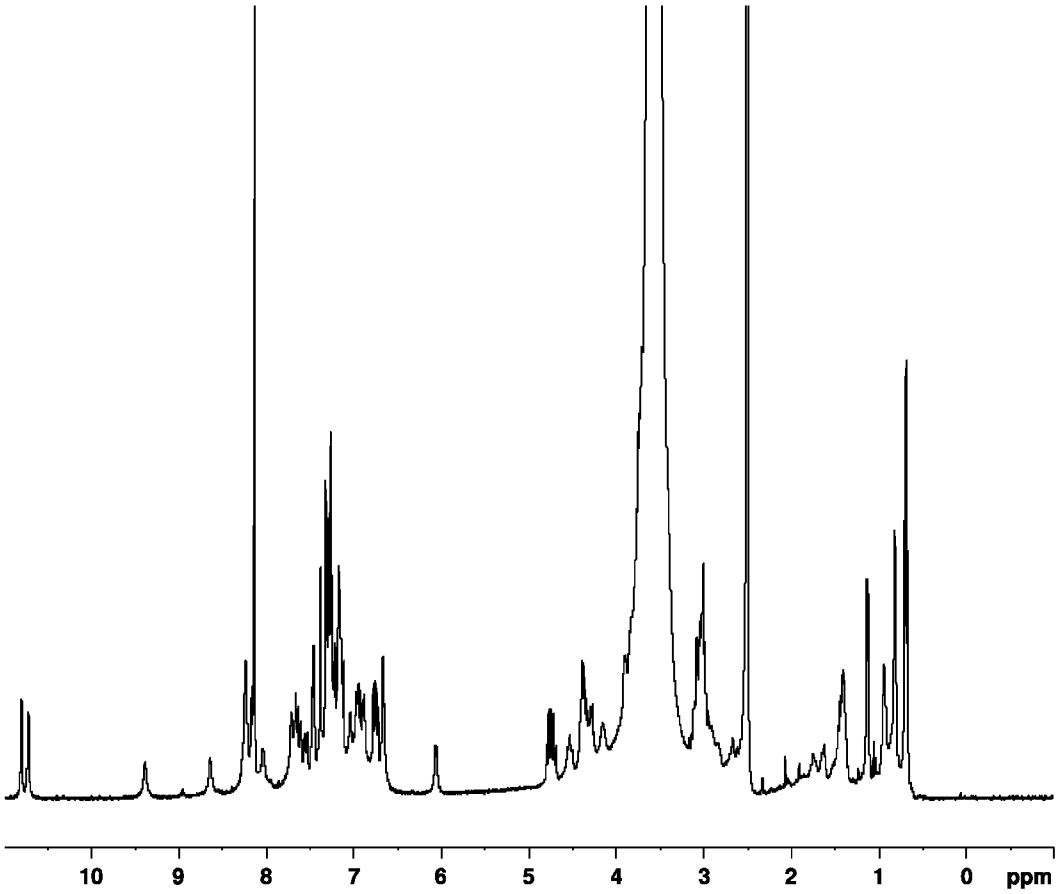


Figure 40

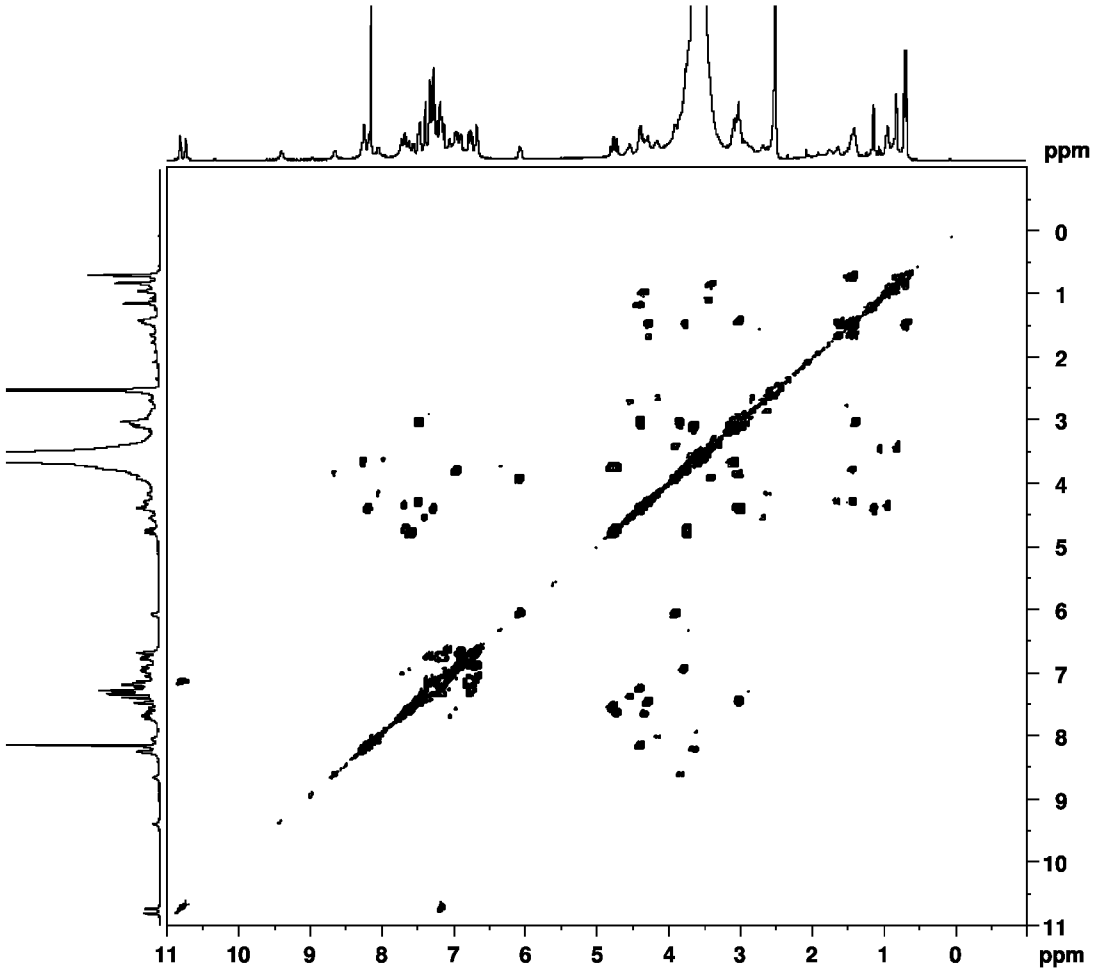


Figure 41

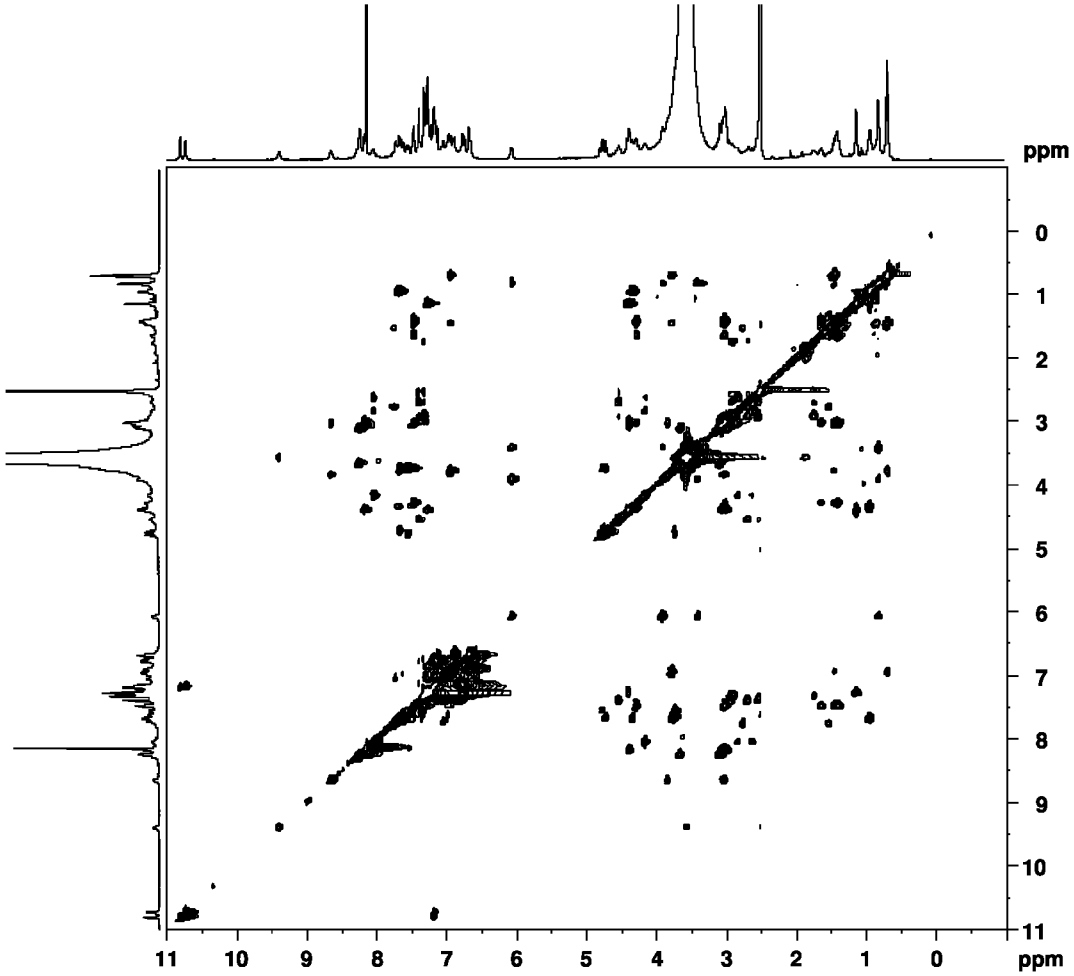


Figure 42

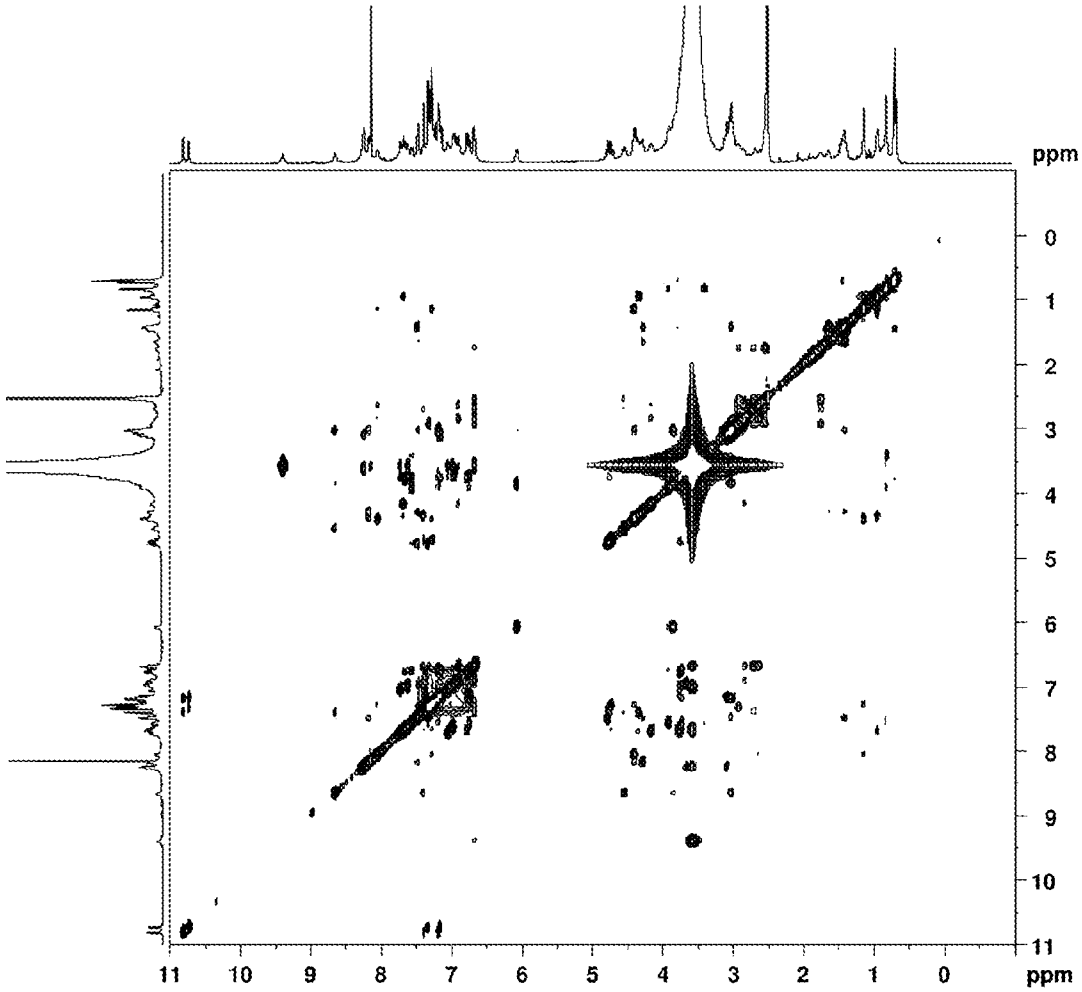


Figure 43

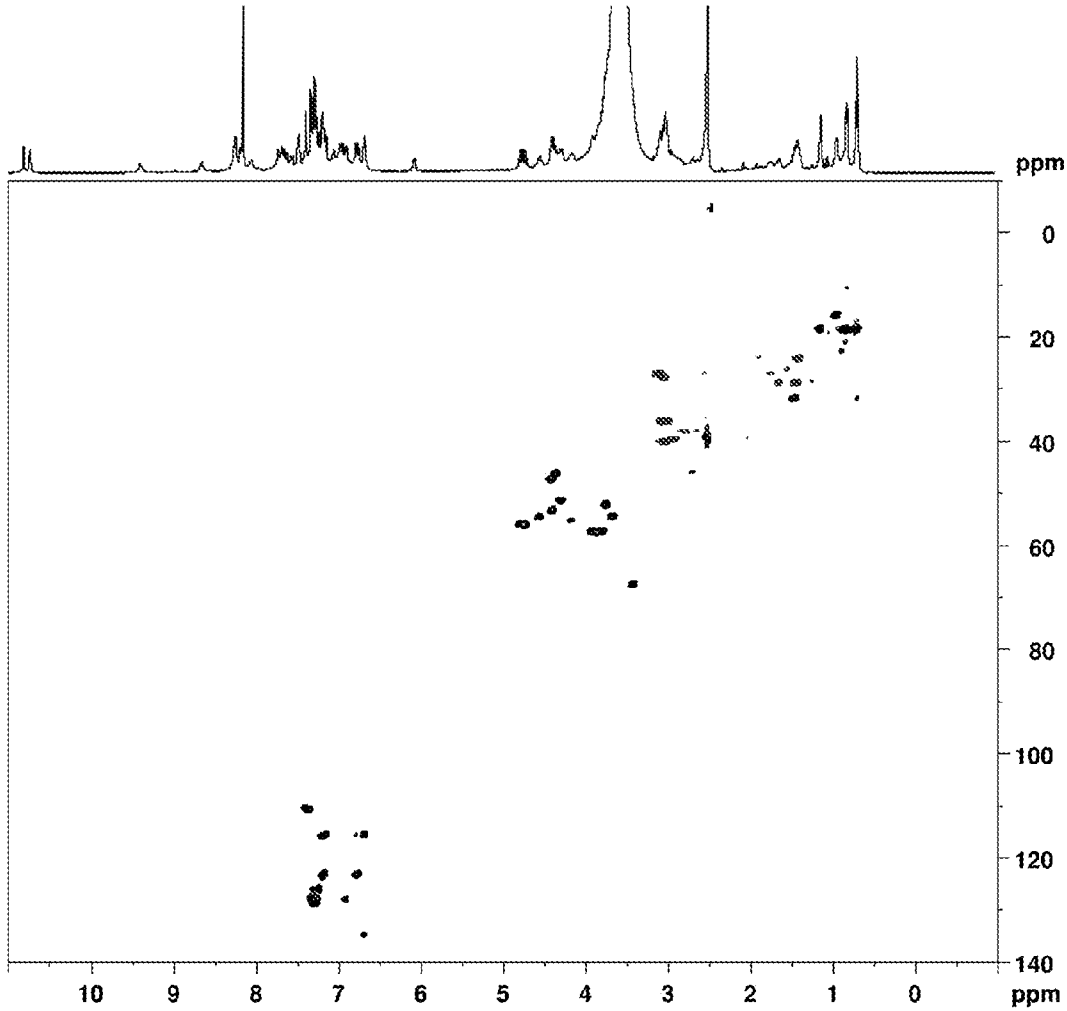


Figure 44

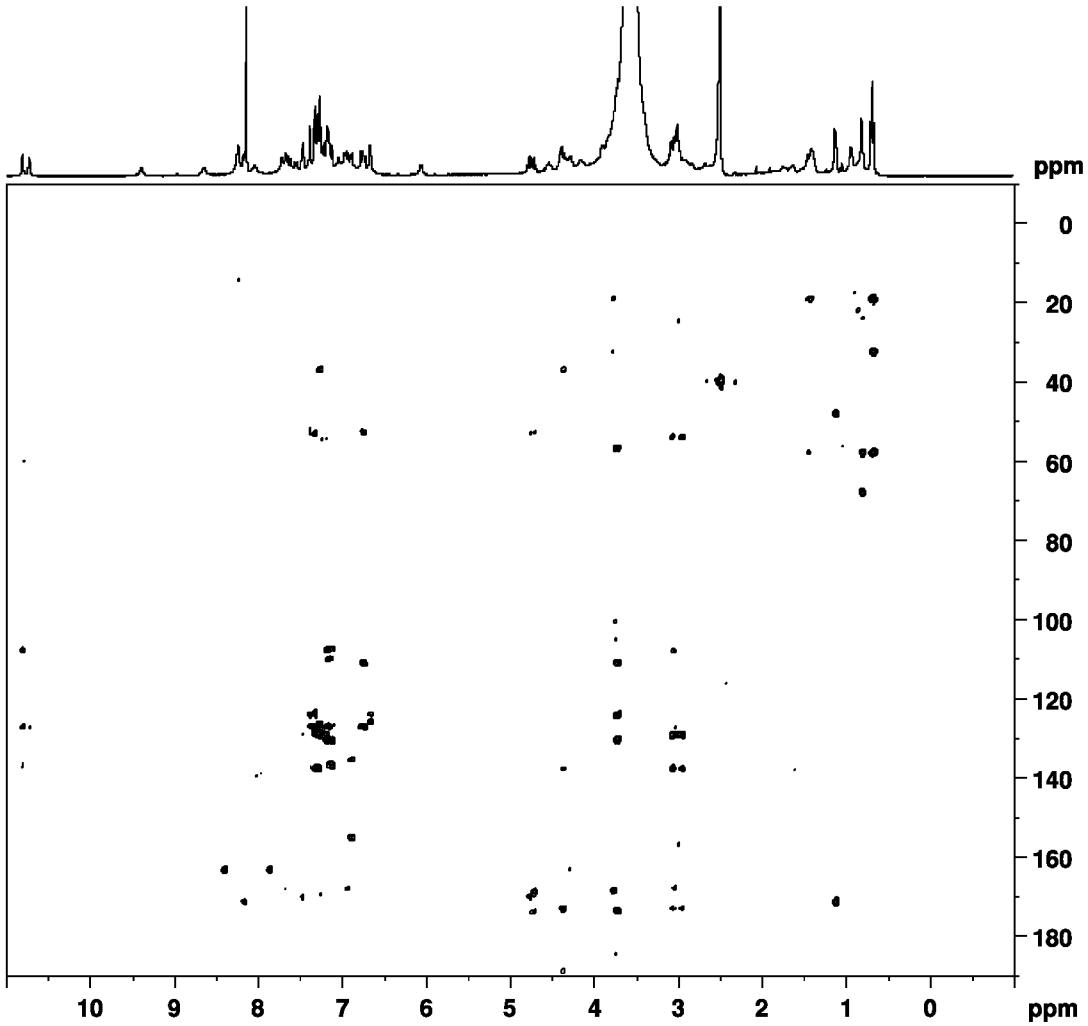


Figure 45

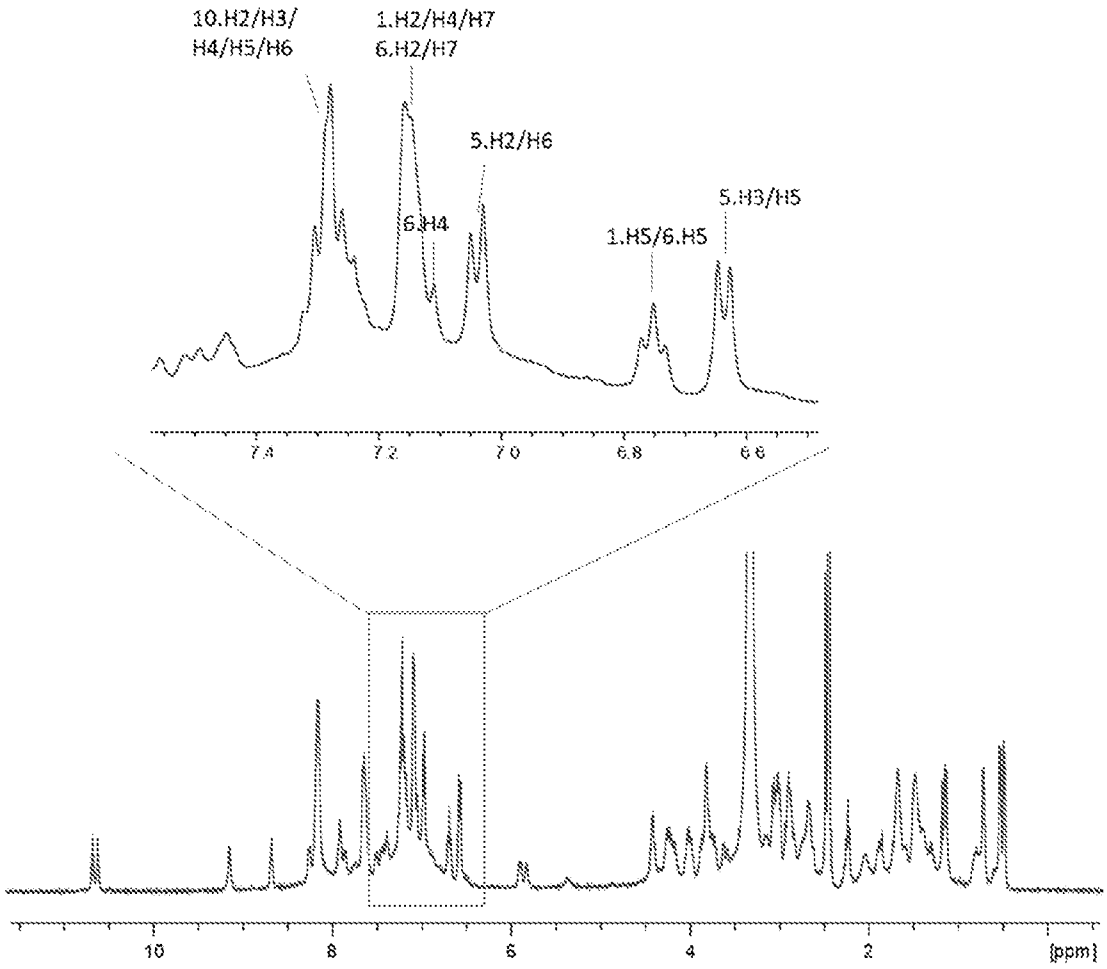

10.H2/H3/
H4/H5/H6
1.H2/H4/H7
6.H2/H7
5.H2/H6
6.H4
1.H5/6.H5
5.H3/H5
7.4
7.2
7.0
6.8
6.6
10
8
6
4
2
(ppm)

Figure 46

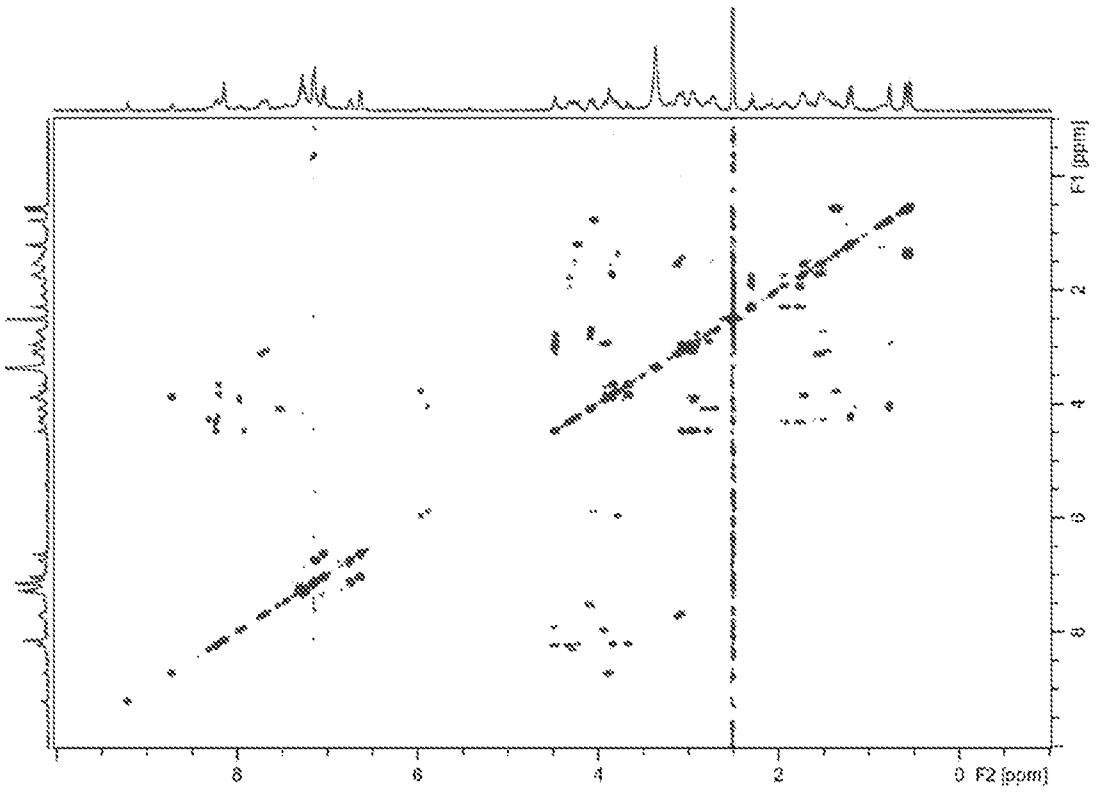


Figure 47

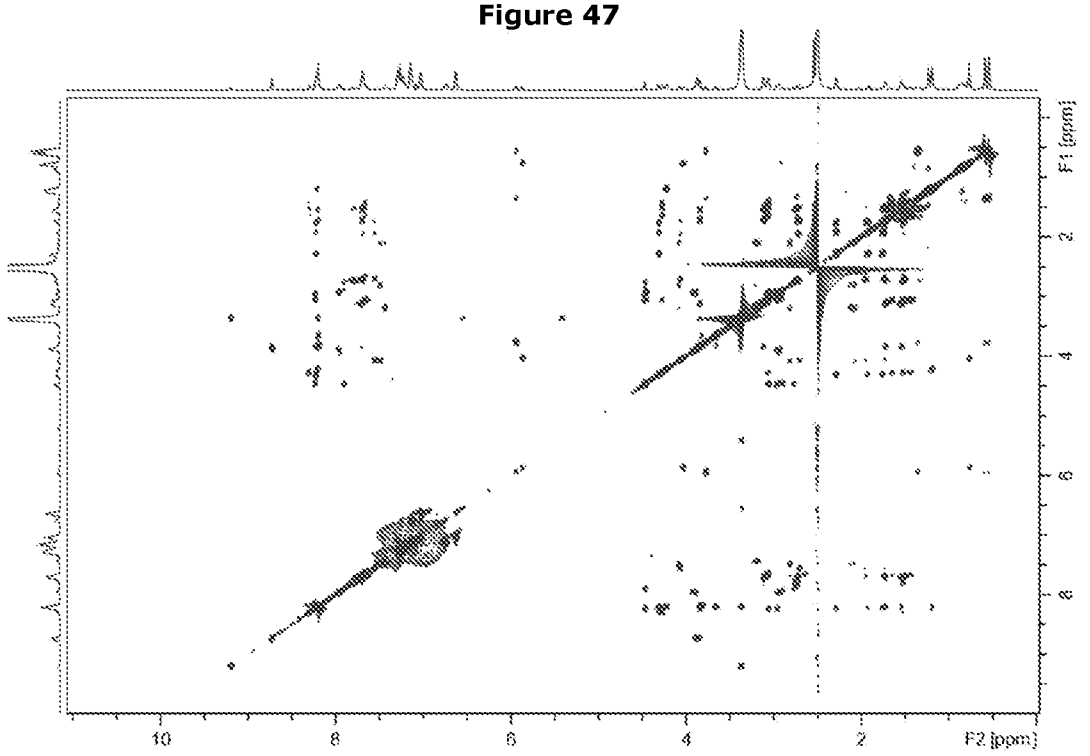


Figure 48

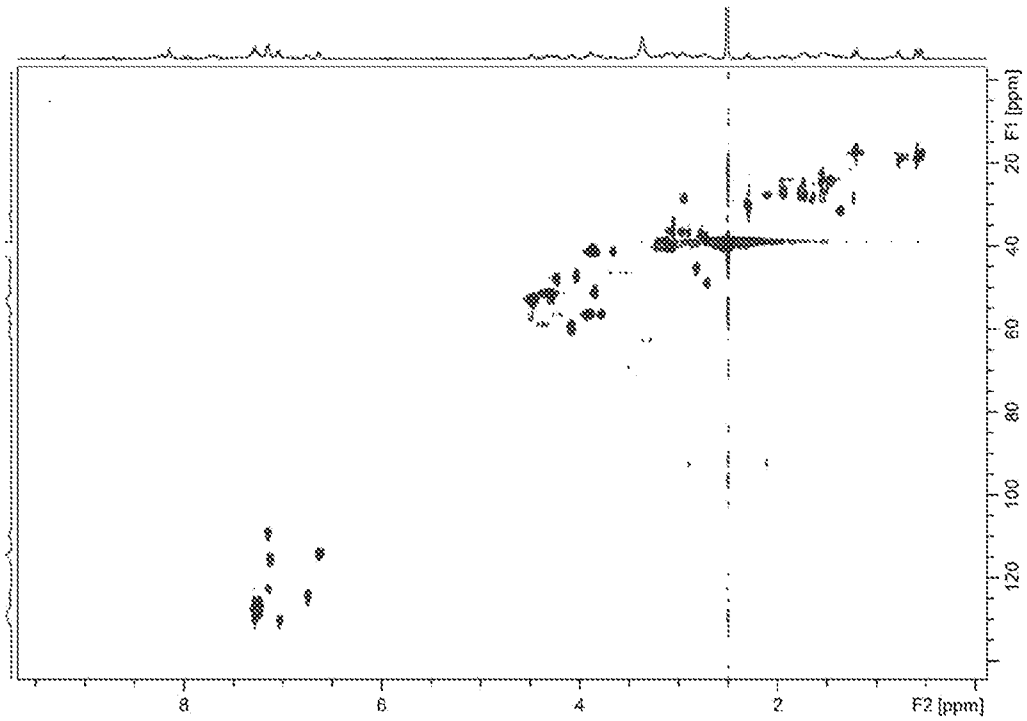


Figure 49

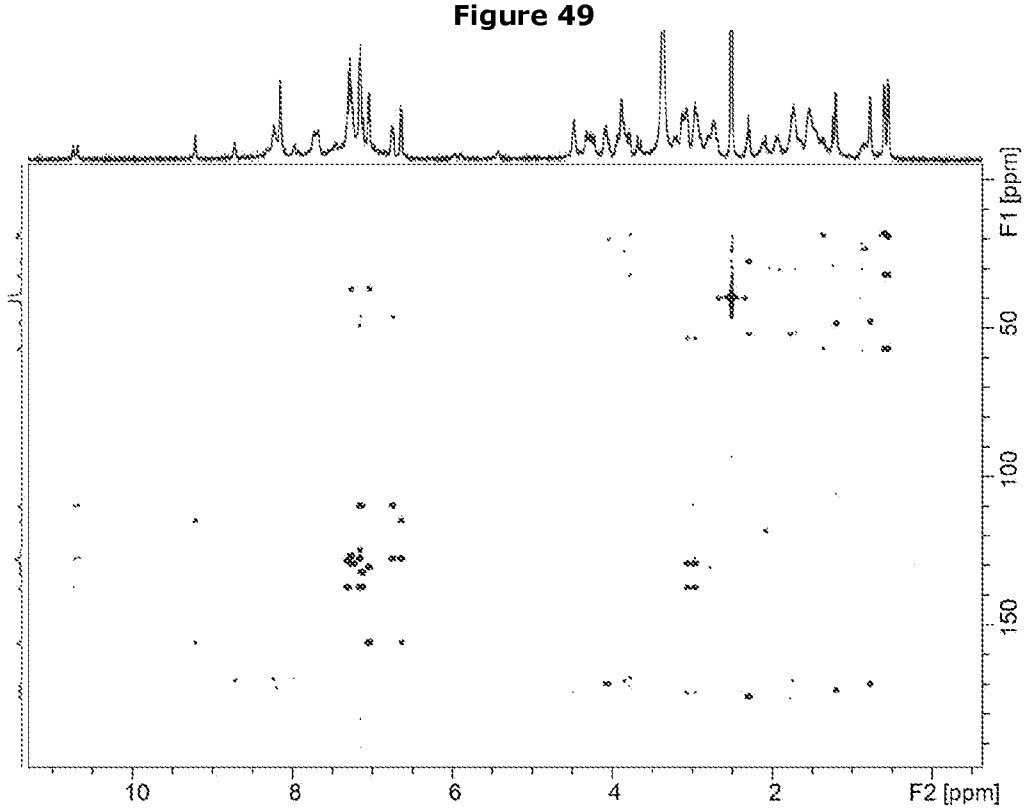


Figure 50

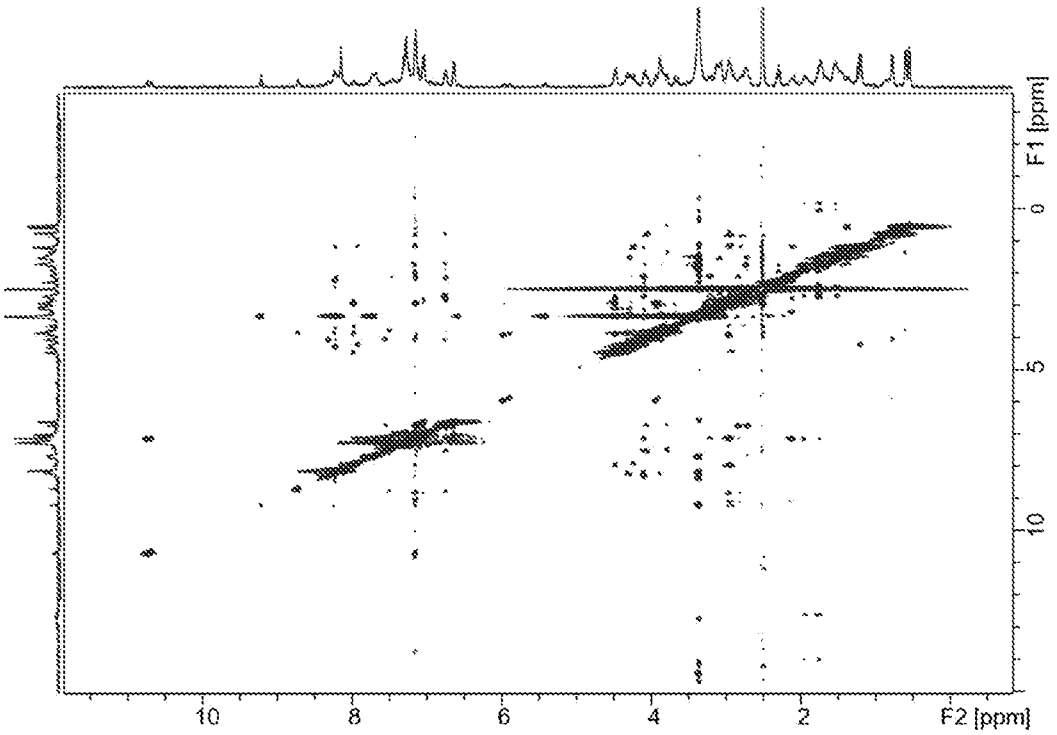


Figure 51

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2023/050524

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed.

   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),

      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2023/050524

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:

   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:

   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:

   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please refer to Supplemental Box (Continuation of Box No. III).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

   1-25 and 43-46

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2023/050524

A. CLASSIFICATION OF SUBJECT MATTER

See Supplemental Box

According to International Patent Classification (IPC)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

FAMPAT, EMBASE, BIOSIS, MEDLINE: Cyclophane, peptides, triceptide, xenorceptide, RiPP, 3-CYFE, and similar terms thereof

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2020/242379 A1 (NATIONAL UNIVERSITY OF SINGAPORE) 3 December 2020 Summary; Figures 1a and 2; SEQ ID NOs: 18-20	1-25 and 43-46
X	HAN Y. ET AL., Substrate specificity and reaction directionality of a three-residue cyclophane forming enzyme PauB. *Chinese Chemical Letters*, 10 June 2022, Vol. 34, No. 1, Article 107589 (Pages 1-5) [Retrieved on 2023-12-07] <DOI: DOI.ORG/10.1016/J.CCLET.2022.06.012> Abstract; Figures 3C, 4A-4C, 5C	1-25 and 43-46
X	SUGIYAMA R. ET AL., The Biosynthetic Landscape of Triceptides Reveals Radical SAM Enzymes That Catalyze Cyclophane Formation on Tyr- and His-Containing Motifs. *J Am Chem Soc.*, 21 June 2022, Vol. 144, No. 26, pages 11580-11593 [Retrieved on 2023-12-07] <DOI: 10.1021/JACS.2C00521> Abstract, Figure 1B, Data Set S3, and bridging paragraph between pages 11582-11583	1-25 and 43-46

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

*Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
07/12/2023 *(day/month/year)*	12/12/2023 *(day/month/year)*

Name and mailing address of the ISA/SG

Intellectual Property Office of Singapore
1 Paya Lebar Link, #11-03
PLQ 1, Paya Lebar Quarter
Singapore 408533

Email: pct@ipos.gov.sg

Authorized officer

Koh Yung Hua (Dr)

IPOS Customer Service Tel. No.: (+65) 6339 8616

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2023/050524

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HE B-B ET AL., Expanded sequence space of radical S-adenosylmethionine-dependent enzyme involved in post-translational macrocyclization. *Research Square*, 29 June 2022 [Retrieved on 2023-12-07] <DOI: DOI.ORG/10.21203/RS.3.RS-1789925/V1> Whole document	-

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SG2023/050524

Note: This Annex lists known patent family members relating to the patent documents cited in this International Search Report. This Authority is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2020/242379 A1	03/12/2020	NONE	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2023/050524

Supplemental Box
(Classification of Subject Matter)

Int. Cl.
C07K 7/08 (2006.01)
C12P 21/04 (2006.01)
C12N 9/00 (2006.01)
A61K 38/10 (2006.01)
A61P 31/00 (2006.01)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2023/050524

Supplemental Box
(Continuation of Box No. III)

This International Searching Authority found multiple inventions in this international application, as follows:

Invention group 1: Claims 1-25 and 43-46

A polypeptide comprising at least two sets of three residue motifs represented by X_1-X_2-X_3, and at least two C-terminus residues, wherein X_1 is selected from tryptophan, phenylalanine, tyrosine, histidine, or an unnatural aromatic amino acid, X_1 and X_3 are connected to form a cyclophane moiety, and at least one of the two C-terminal residues is an aromatic residue. ***Specific polypeptides and medical uses for these peptides*** are also encompassed.

Invention group 2: Claims 26-38

A method of producing a polypeptide in a host cell, comprising introducing a ***nucleic acid expressing a rSAM/rSPASM maturase, a protease, a transporter, a protease/transporter, and a precursor polypeptide***, wherein the precursor polypeptide comprising at least two sets of three residue motifs represented by X_1-X_2-X_3, and at least two C-terminus residues, wherein X_1 is selected from tryptophan, phenylalanine, tyrosine, histidine, or an unnatural aromatic amino acid, X_1 and X_3 are connected to form a cyclophane moiety, and at least one of the two C-terminal residues is an aromatic residue.

Invention group 3: Claims 39 and 41-42

A method of producing a polypeptide comprising the ***provision of a precursor polypeptide and a enzyme, such as a rSAM/SPASM maturase***, wherein the precursor polypeptide comprising at least two sets of three residue motifs represented by X_1-X_2-X_3, and at least two C-terminus residues, wherein X_1 is selected from tryptophan, phenylalanine, tyrosine, histidine, or an unnatural aromatic amino acid, X_1 and X_3 are connected to form a cyclophane moiety, and at least one of the two C-terminal residues is an aromatic residue.

Invention group 4: Claim 40

A method of synthesising a polypeptide comprising ***coupling a pre-sequence peptide to a support, adding N-protected amino acids to the precursor polypeptide, cleaving the precursor polypeptide, and synthetically or enzymatically connecting the X_1 and X_3 residues of the peptide to form a cyclophane moiety***.

Please refer to **Box No. IV** of Written Opinion of The International Searching Authority (Form PCT/ISA/237) for detailed explanation.