

A proof-reading mechanism for non-proteinogenic amino acid incorporation into glycopeptide antibiotics

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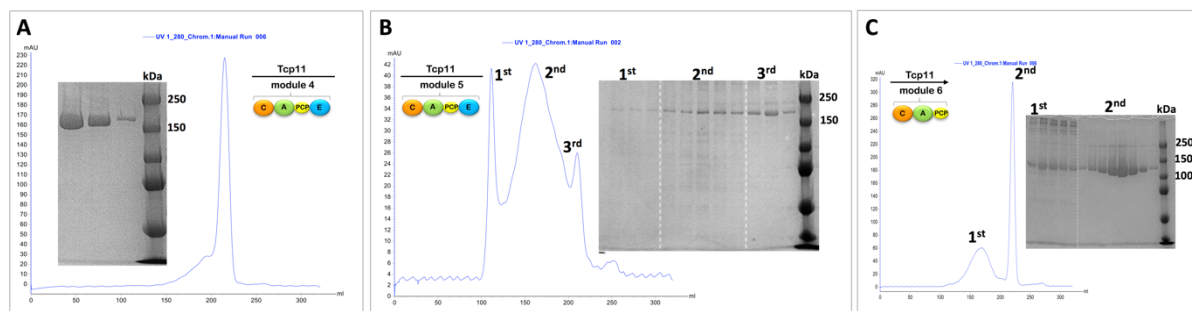
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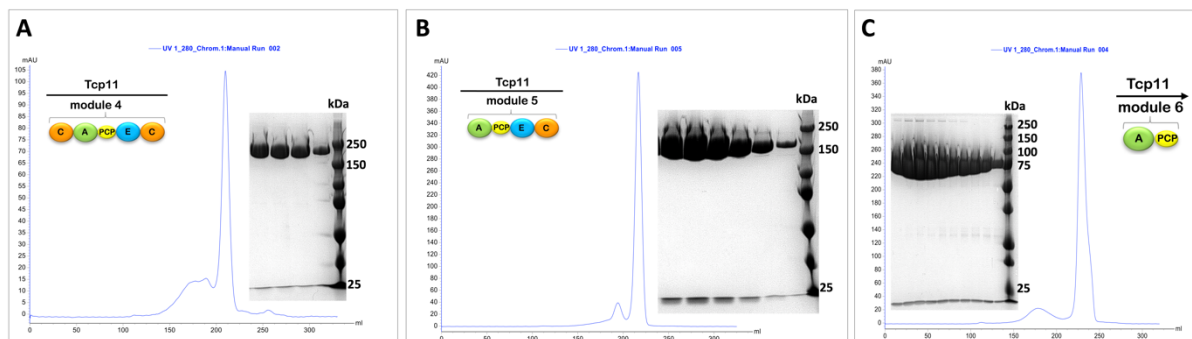
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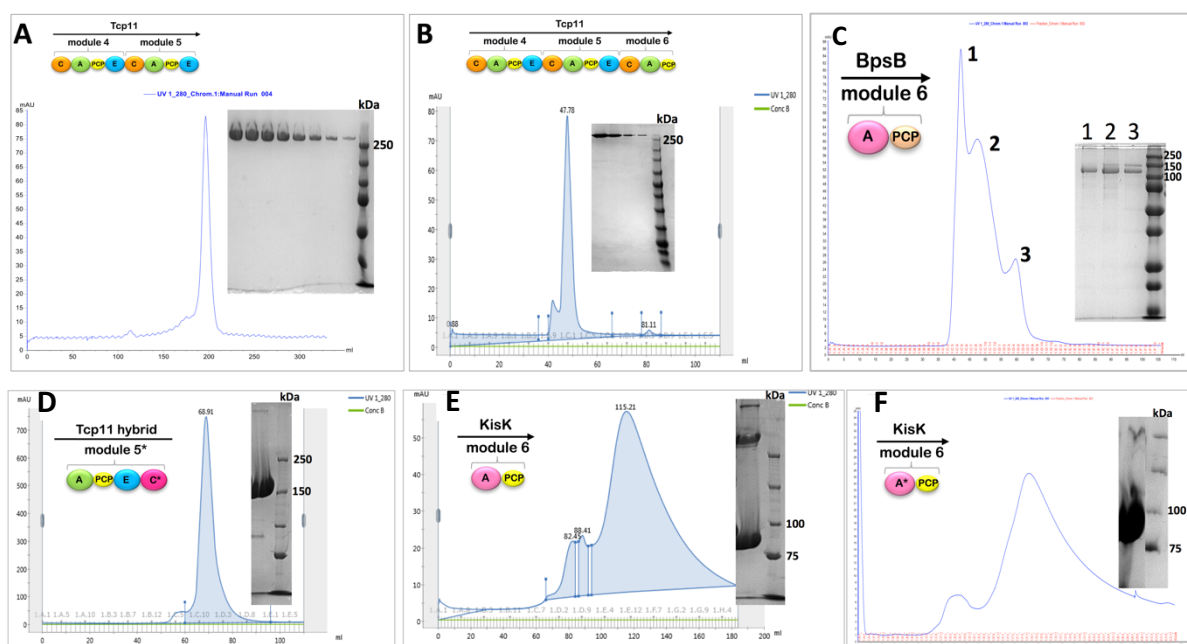
SI Figures



SI Figure S1. Results of gel filtration purification of three Tcp11 modules divided according to traditional module (E/C) division. A) Module 4 (C-A-PCP-E, GB1 fusion, 170 kDa). B) Module 5 (C-A-PCP-E, GB1 fusion, 171 kDa). Three peaks after gel filtration indicate incorrect folding and protein aggregation. C) Module 6 (C-A-PCP, GB1 fusion, 124 kDa). The first peak indicates aggregated protein fraction that was separated from monomeric protein (second peak). Isolated protein yields: 7 mg of module 4, ~700 ng scale of module 5 and 9 mg of module 6 were isolated from 10 L of bacterial growth media, respectively.

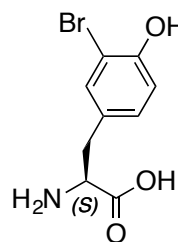


SI Figure S2. Results of gel filtration purification of alternate architecture Tcp11 modules. A) Module 4 (C-A-PCP-E-C, GB1 fusion, 218 kDa). B) Module 5 (A-PCP-E-C, GB1 fusion, 171 kDa). C) Module 6 (A-PCP, GB1 fusion, 74 kDa). Isolated protein yields: 4.5 mg of module 4, 15 mg of module 5 and 20 mg of module 6 were isolated from 10 L of bacterial growth media, respectively.



SI Figure S3. Results of gel filtration purification from other NRPS proteins analysed in this study. A) Tcp11 di-module 4-5 (C-A-PCP-E-C-A-PCP-E, GB1 fusion, 329 kDa), B) Full length tri-module 4-5-6 Tcp11 protein (C-A-PCP-E-C-A-PCP-E-C-A-PCP, GB1 fusion, 441 kDa). C) BpsB module 6 (A-PCP, MBP fusion, 113 kDa). Three peaks indicate protein aggregation. Due to low protein yield, both monomeric (3rd peak) and dimeric (2nd peak) fractions were used for biochemical characterisation. D) Tcp11 module 5 hybrid (A-PCP-E-C*, GB1 fusion, 172 kDa). E) KisK module 6 (A-PCP, GB1 fusion, 78 kDa). F) KisK module 6 A domain double mutant (A*-PCP, GB1 fusion, 78 kDa). Isolated protein yields: 1 mg of Tcp11 di-module 4-5, < 1mg of full length Tcp11, 1mg of BpsB module 6, 20 mg of Tcp11 module 5 hybrid, 5.4 mg of KisK module 6 and 6.5 mg of KisK module 6 A domain double mutant were isolated from 10 L of bacterial growth media, respectively.

SI Figure S4. Synthesis and characterisation of Br-Tyr (**7**)



Chemical Formula: C₉H₁₀BrNO₃

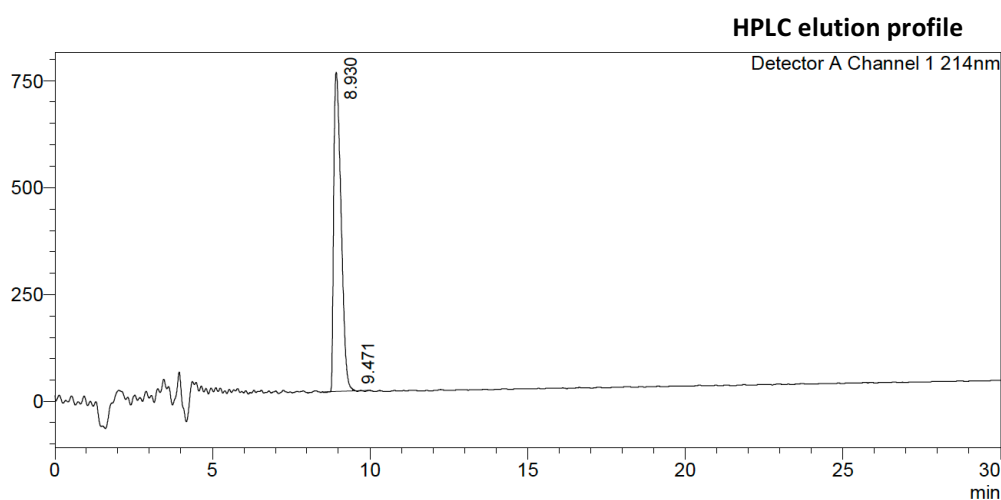
Exact Mass: 258.98 Da

Retention time: 8.930 min (99%).

MS (ESI+): m/z calcd C₉H₁₀BrNO₃ 259.00, found m/z C₉H₁₁ClNO₃⁺ 260.00,

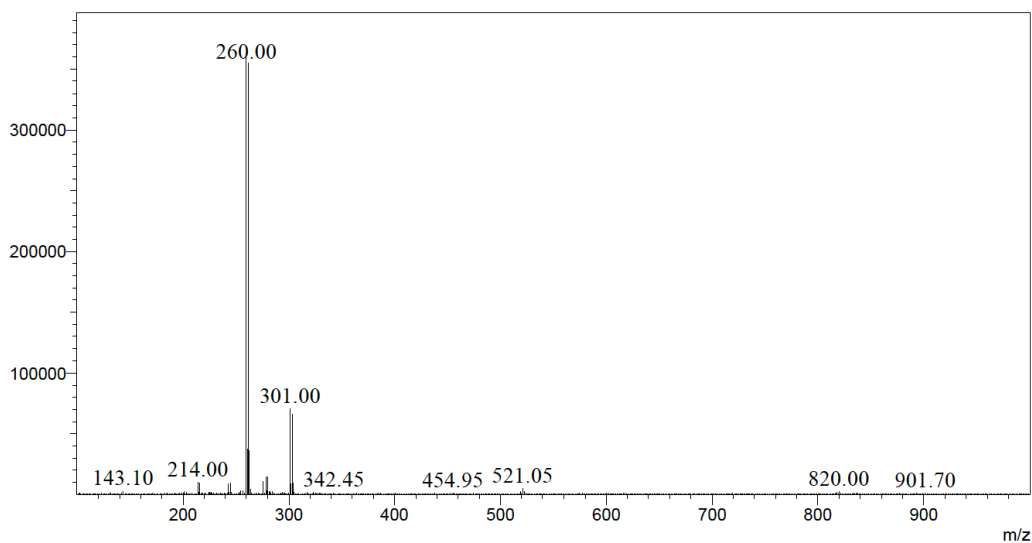
C₁₁H₁₄BrN₂O₃⁺ (ACN adduct) 301.00.

(400Hz, CD₃N/D₂O): δ = 2.9 ppm (dd, 1H, J1 = 6.6MHz, J2 = 8.1MHz), 3.2 ppm (dd, 1H, J1 = 9.8 MHz, J2 = 4.9MHz), 3.9 ppm (m, 1H), 6.9 (d, 1H), 7.1 (dd, 1H), 7.4 (d, 1H).

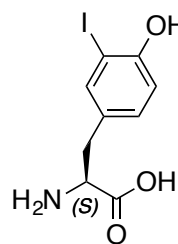


Line#:1 R.Time:0.000(Scan#:1)
MassPeaks:901
RawMode:Averaged 8.433-9.933(507-597) BasePeak:260.00(359445)
BG Mode:None Segment 1 - Event 1

MS spectrum in positive mode



SI Figure S5. Synthesis and characterisation of l-Tyr (8)



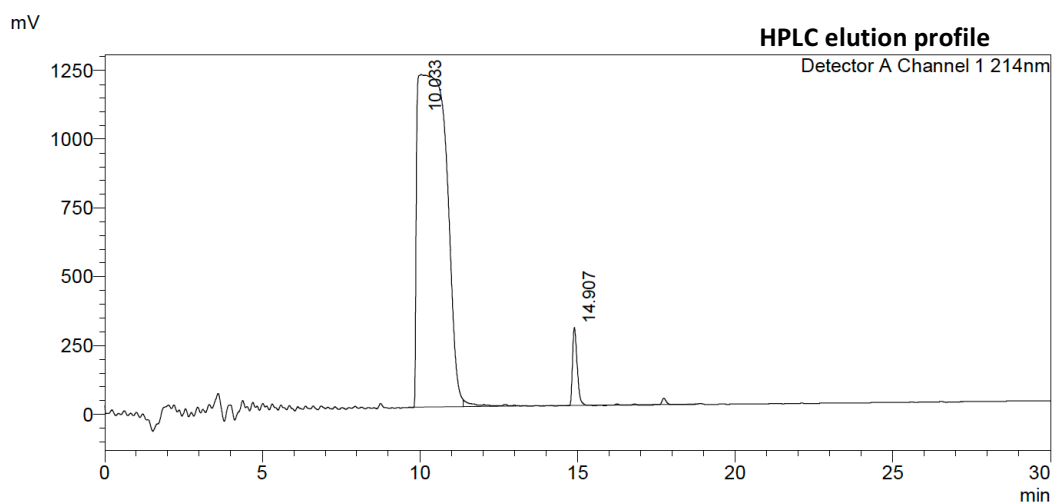
Chemical Formula: C₉H₁₀INO₃

Exact Mass: 306.97 Da

Retention time: 10.330 min (95%).

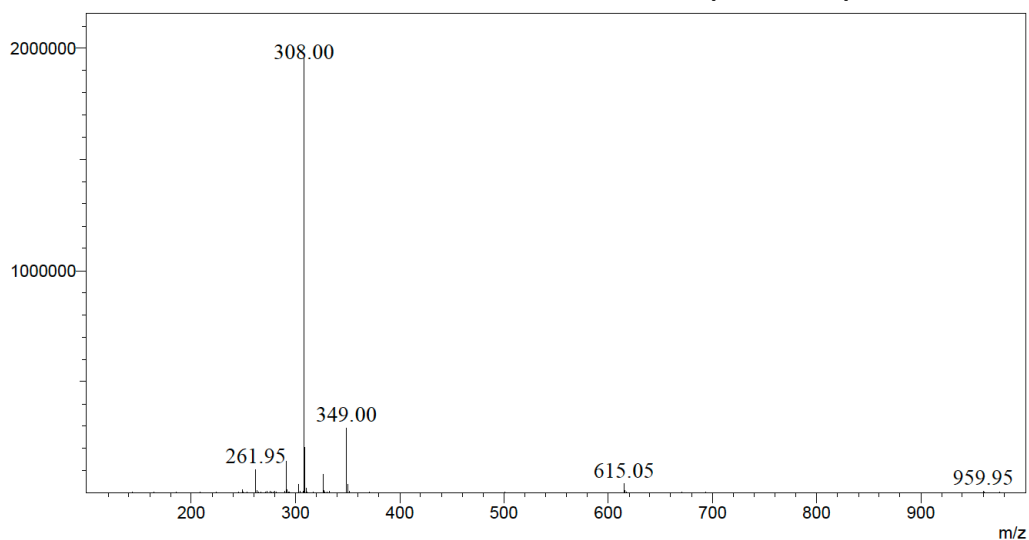
MS (ESI+): m/z calcd C₉H₁₀INO₃ 307.00, found m/z C₉H₁₁ClNO₃⁺ 308.00, C₁₁H₁₄IN₂O₃⁺ (ACN adduct) 349.00.

(400Hz, CD₃N/D₂O): δ = 3.0 ppm (dd, 1H, J1 = 6.6MHz, J2 = 8.0MHz), 3.2 ppm (dd, 1H, J1 = 9.6 MHz, J2 = 5.0MHz), 4.0 ppm (m, 1H), 6.8 (d, 1H), 7.1 (dd, 1H), 7.6 (d, 1H).

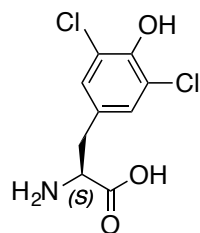


Line#:1 R.Time:10.167(Scan#:611)
MassPeaks:901
RawMode:Averaged 9.300-11.900(559-715) BasePeak:308.00(1956028)
BG Mode:None Segment 1 - Event 1

MS spectrum in positive mode



SI Figure S6. Synthesis and characterisation of di-Cl-Tyr (9)



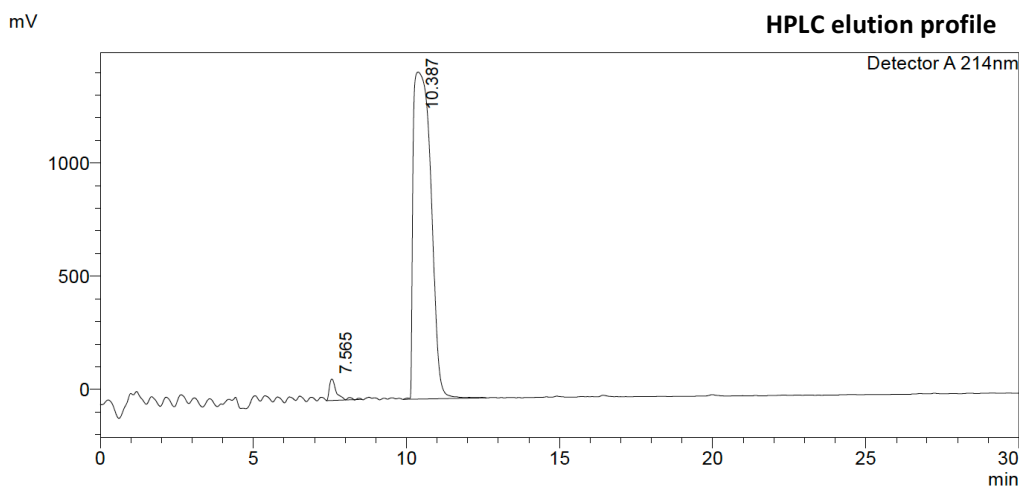
Chemical Formula: C₉H₉Cl₂NO₃

Exact Mass: 249.00 Da

Retention time: min 10.387 (97%).

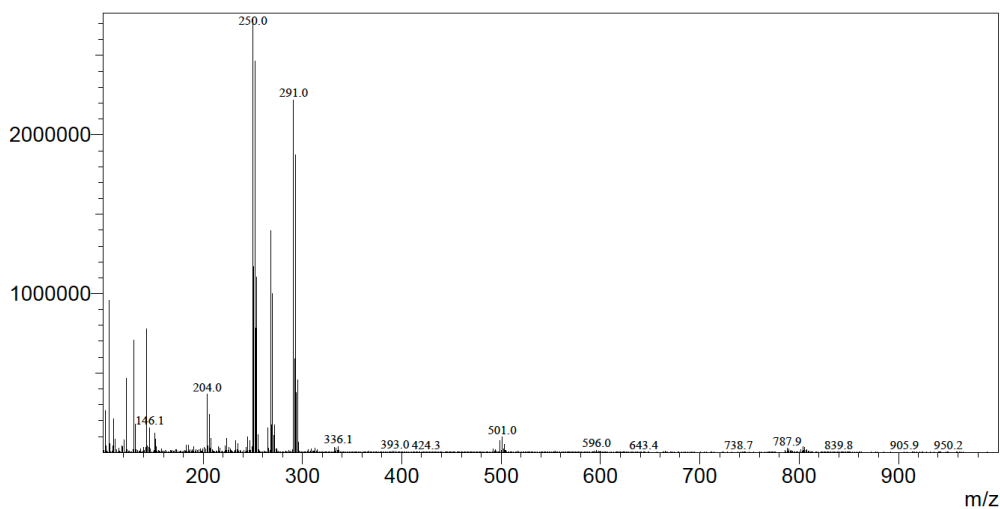
MS (ESI+): m/z calcd C₉H₉Cl₂NO₃ 249.00, found m/z C₉H₁₀Cl₂NO₃⁺ 250.0, C₁₁H₁₄ClN₂O₃⁺ (ACN adduct) 291.0.

(400MHz, CD₃N/D₂O): δ = 3.0 ppm (dd, 1H, J1 = 14.7MHz, J2 = 7.9MHz), 3.2 ppm (dd, 1H, J1 = 14.7 MHz, J2 = 5.0MHz), 3.9 ppm (dd, 1H, J1 = 7.9 MHz, J2 = 5.1MHz), 7.2 ppm (s, 2H).

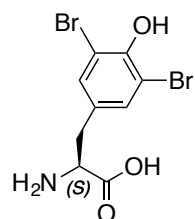


Line#:1 R.Time:----(Scan#:----)
MassPeaks:901
RawMode:Averaged 9.267-12.400(557-745) BasePeak:250.0(2738512)
BG Mode:None Segment 1 - Event 1

MS spectrum in positive mode



SI Figure S7. Synthesis and characterisation of di-Br-Tyr (10)



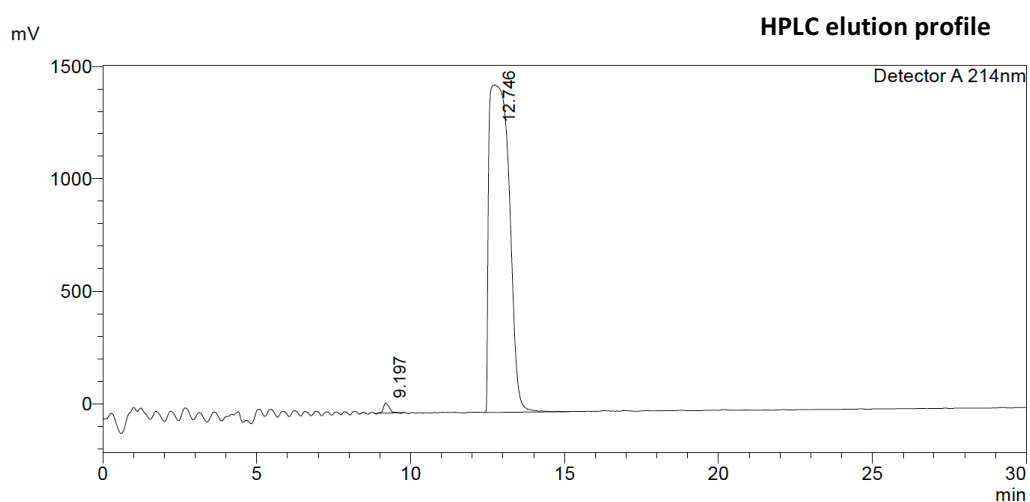
Chemical Formula: $C_9H_9Br_2NO_3$

Exact Mass: 336.89 Da

Retention time: 12.746min (99%).

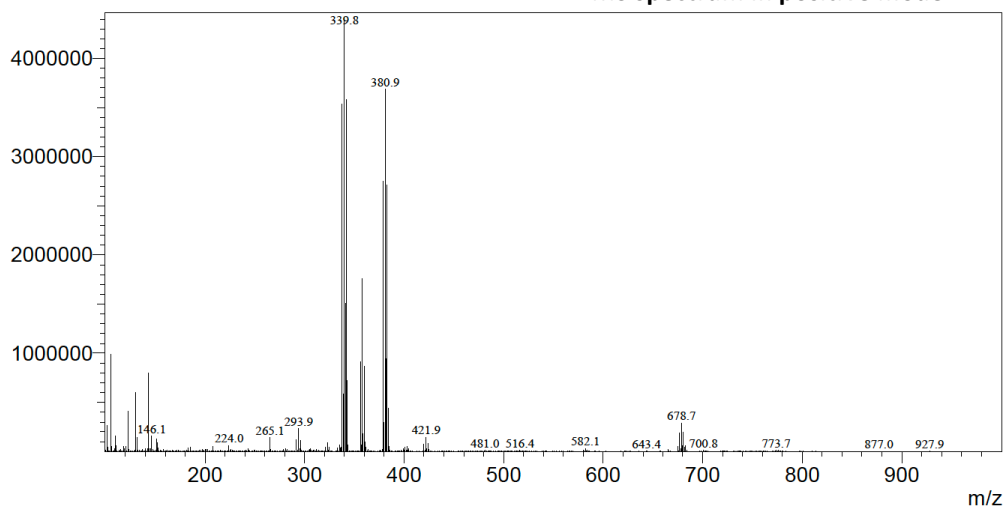
MS (ESI+): m/z calcd $C_9H_9Br_2NO_3$ 338.8, found m/z $C_9H_{10}Br_2NO_3^+$ 339.8, $C_{11}H_9Br_2N_2O_3^+$ (ACN adduct) 380.9.

(400MHz, CD_3N/D_2O): δ = 3.0 ppm (dd, 1H, $J_1 = 14.7$ MHz, $J_2 = 7.8$ MHz), 3.2 ppm (dd, 1H, $J_1 = 14.7$ MHz, $J_2 = 5.2$ MHz), 4.0 ppm (dd, 1H, $J_1 = 7.8$ MHz, $J_2 = 5.2$ MHz), 7.4 ppm (s, 2H).

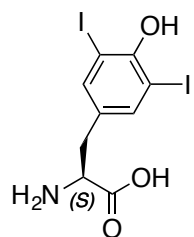


Line#:1 R.Time:----(Scan#:----)
MassPeaks:901
RawMode:Averaged 12.000-14.167(721-851) BasePeak:339.8(4419553)
BG Mode:None Segment 1 - Event 1

MS spectrum in positive mode



SI Figure S8. Synthesis and characterisation of di-I-Tyr (11)



Chemical Formula: C₉H₉I₂NO₃

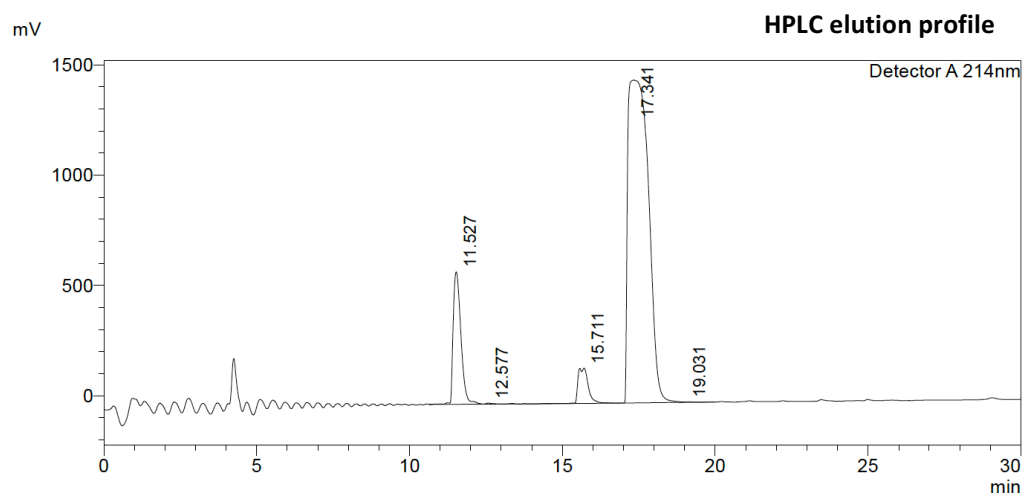
Exact Mass: 432.87 Da

Retention time: 17.341min (82%).

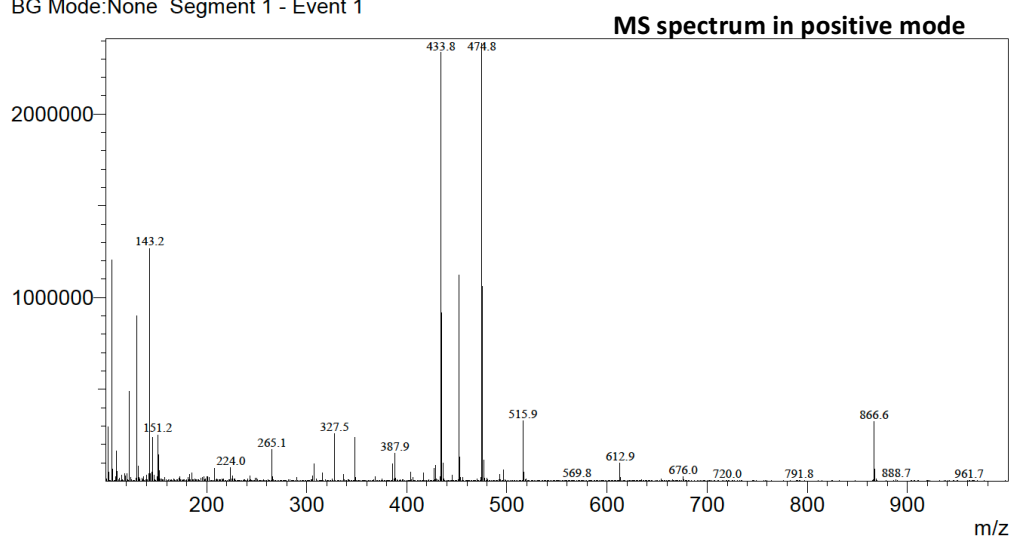
MS (ESI+): m/z calcd C₉H₉I₂NO₃ 432.8, found m/z C₉H₁₀I₂NO₃⁺ 433.8,

C₁₁H₉I₂N₂O₃⁺ (ACN adduct) 474.8.

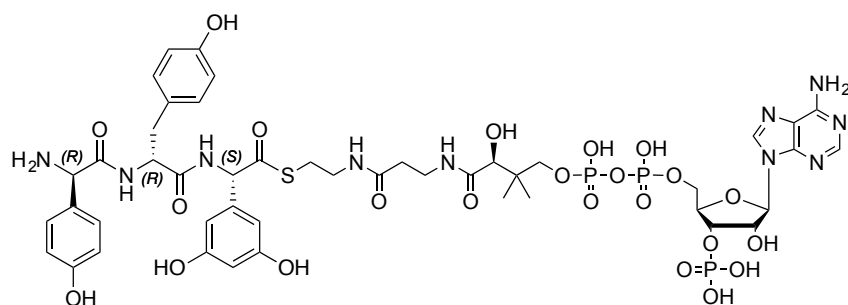
(400MHz, CD₃N/D₂O): δ = 2.9 ppm (dd, 1H, J1 = 14.7MHz, J2 = 7.8MHz), 3.1 ppm (dd, 1H, J1 = 14.7 MHz, J2 = 5.3MHz), 4.0 ppm (dd, 1H, J1 = 7.8MHz, J2 = 5.3MHz), 7.7ppm (s, 2H).



Line#:1 R.Time:----(Scan#:----)
MassPeaks:901
RawMode:Averaged 15.133-20.267(909-1217) BasePeak:474.8(2386095)
BG Mode:None Segment 1 - Event 1



SI Figure S9. Synthesis and characterisation of NH₂-(D)-Hpg-(D)-Tyr-(L)-Dpg-CoA tripeptide (3T-CoA).



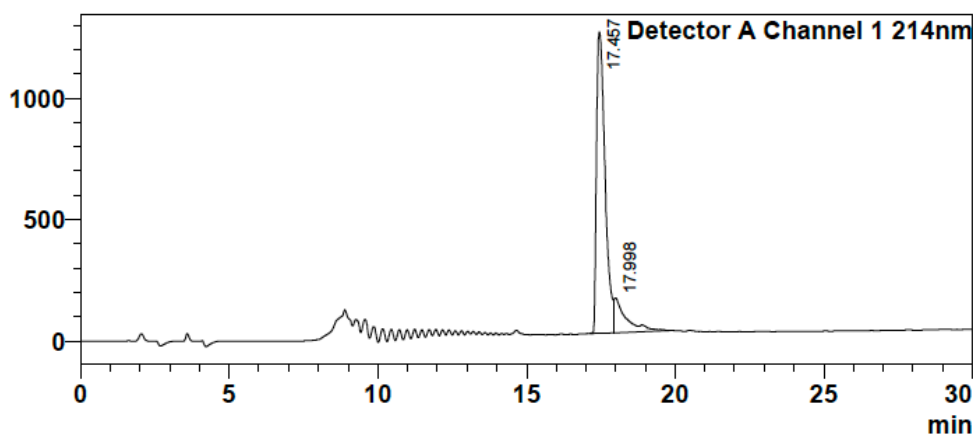
Chemical Formula: C₄₆H₅₉N₁₀O₂₃P₃S

Exact Mass: 1244.27 Da

Retention time: 17.457 min (86%); 5-35% to ACN gradient over 30 minutes

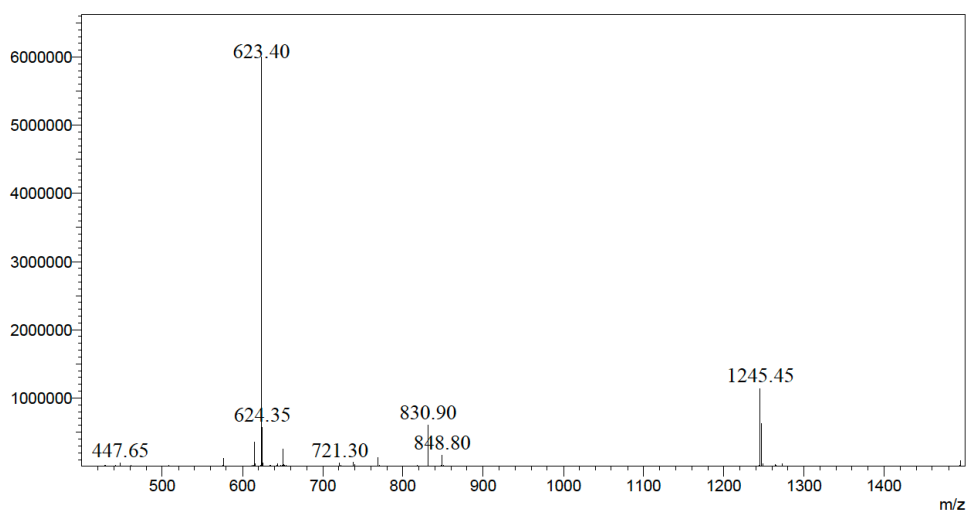
MS (ESI): *m/z* calcd C₄₆H₅₉N₁₀O₂₃P₃S 1244.45, found [M+H]⁺ *m/z* C₄₆H₆₀N₁₀O₂₃P₃S⁺ 1245.45, [M+H]²⁺ C₄₆H₆₀N₁₀O₂₃P₃S²⁺ 623.40.

HPLC elution profile

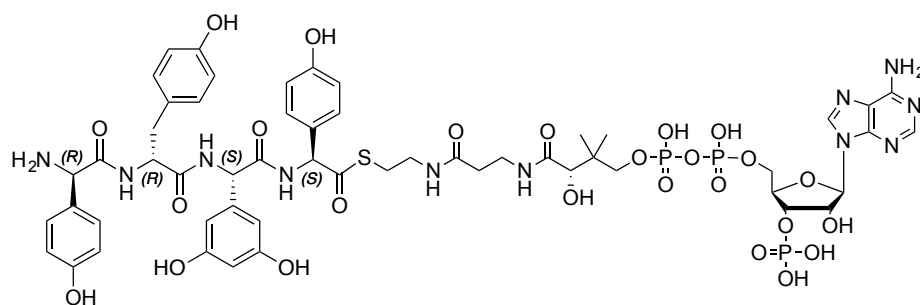


Line#:1 R.Time:17.467(Scan#:1049)
 MassPeaks:1180
 RawMode:Single 17.467(1049) BasePeak:623.40(5995040)
 BG Mode:None Segment 1 - Event 1

MS spectrum in positive mode



SI Figure S10. Synthesis and characterisation of NH₂-(D)-Hpg-(D)-Tyr-(L)-Dpg-(L)-Hpg-CoA tetrapeptide (**4T-CoA**).

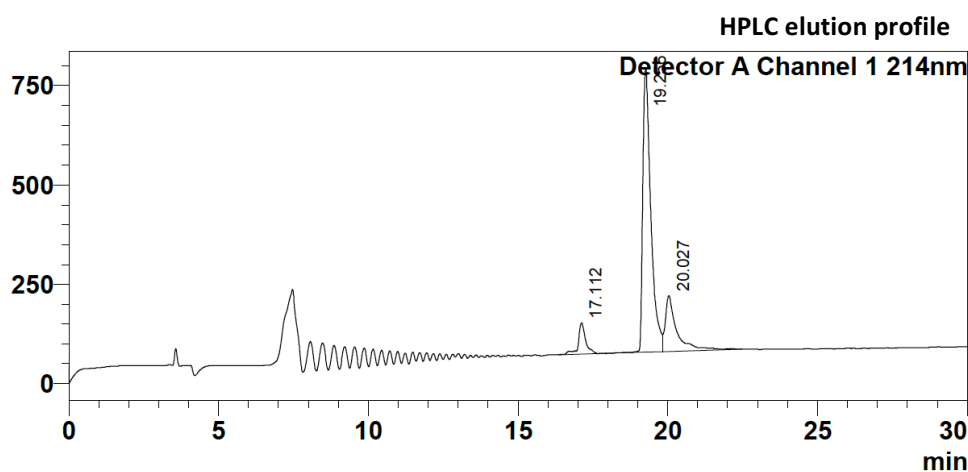


Chemical Formula: C₅₄H₆₆N₁₁O₂₅P₃S

Exact Mass: 1393.32 Da

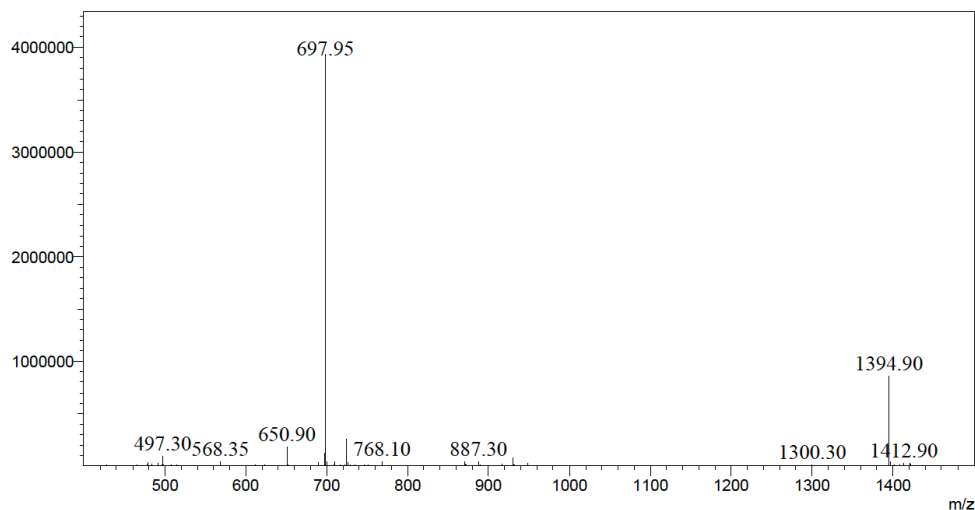
Retention time: 19.256 min (72%); 5-35% to ACN gradient over 30 minutes

MS (ESI): *m/z* calcd C₅₄H₆₆N₁₁O₂₅P₃S 1393.9, found [M+H]⁺ *m/z* C₅₄H₆₇N₁₁O₂₅P₃S⁺ 1394.9, [M+H]²⁺ C₅₄H₆₈N₁₁O₂₅P₃S²⁺ 697.95.

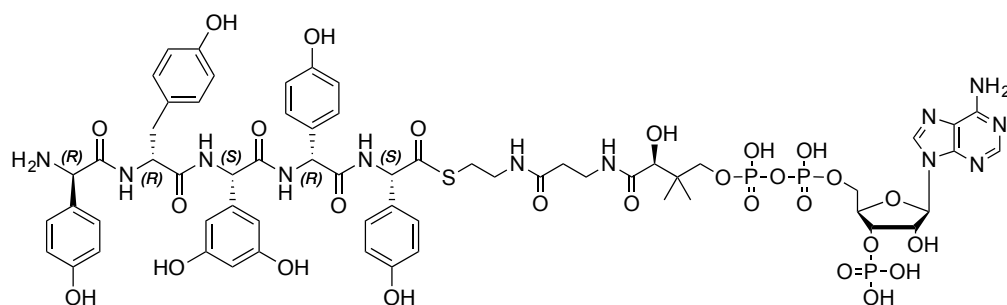


Line#:1 R.Time:19.267(Scan#:1157)
 MassPeaks:1173
 RawMode:Single 19.267(1157) BasePeak:697.95(3931709)
 BG Mode:None Segment 1 - Event 1

MS spectrum in positive mode



SI Figure S11. Synthesis and characterisation of NH₂-(D)-Hpg-(D)-Tyr-(L)-Dpg-(D)-Hpg-(L)-Hpg-CoA pentapeptide (**5T-CoA**).



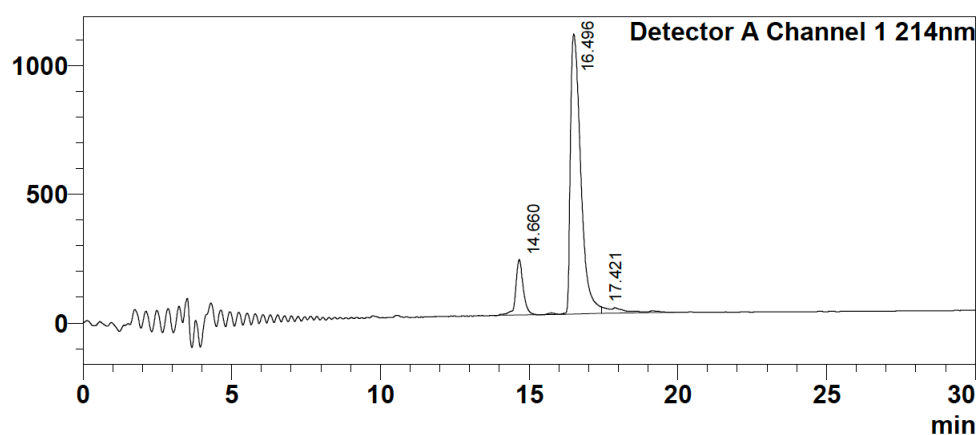
Chemical Formula: C₆₂H₇₃N₁₂O₂₇P₃S

Exact Mass: 1542.36 Da

Retention time: 16.496 min (84%); 10-40% to ACN gradient over 30 minutes

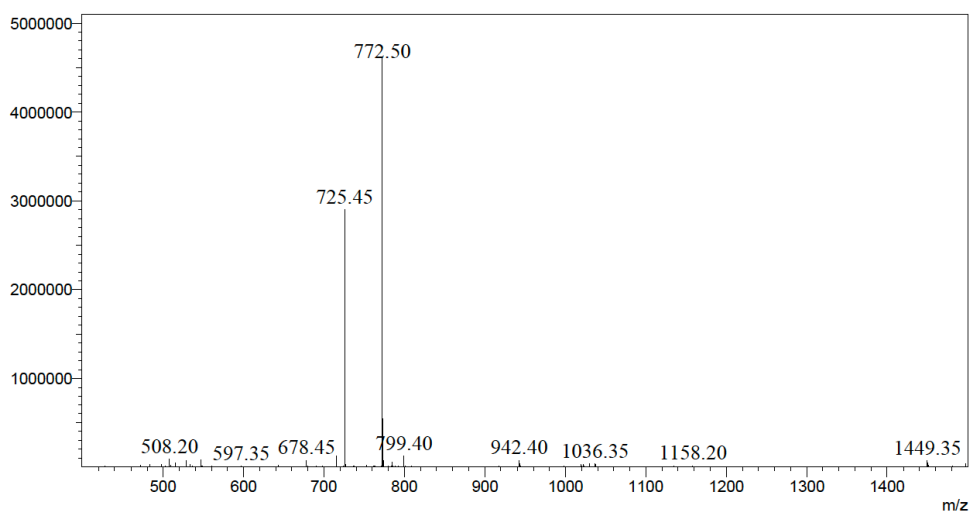
MS (ESI): *m/z* calcd C₆₂H₇₃N₁₂O₂₇P₃S 1543.00, found [M+H]⁺ *m/z* C₆₂H₇₅N₁₂O₂₇P₃S²⁺ 772.5.

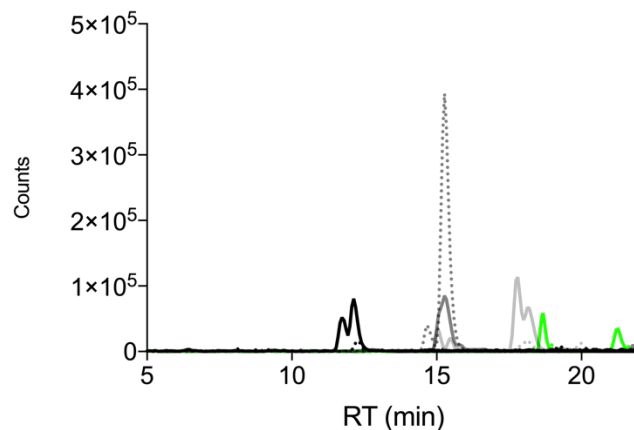
HPLC elution profile



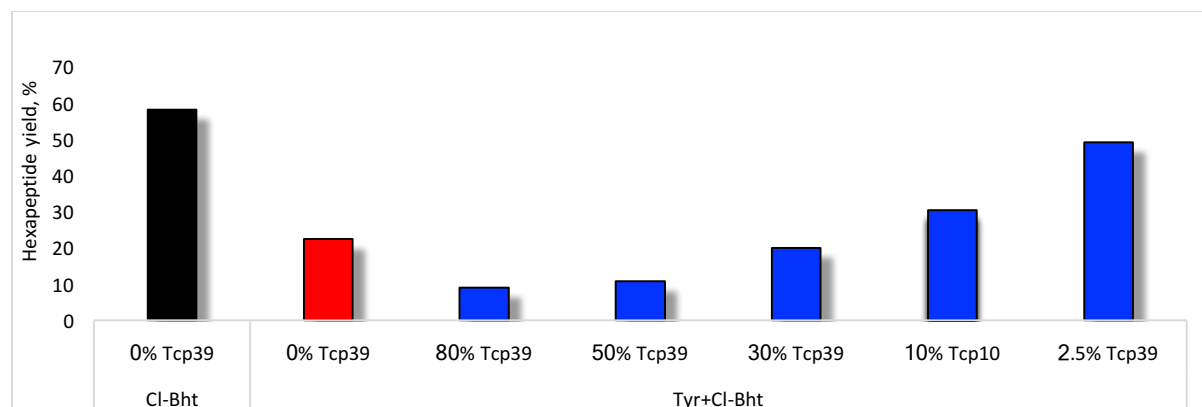
Line#:1 R.Time:16.533(Scan#:993)
 MassPeaks:1192
 RawMode:Single 16.533(993) BasePeak:772.50(4619733)
 BG Mode:None Segment 1 - Event 1

MS spectrum in positive mode

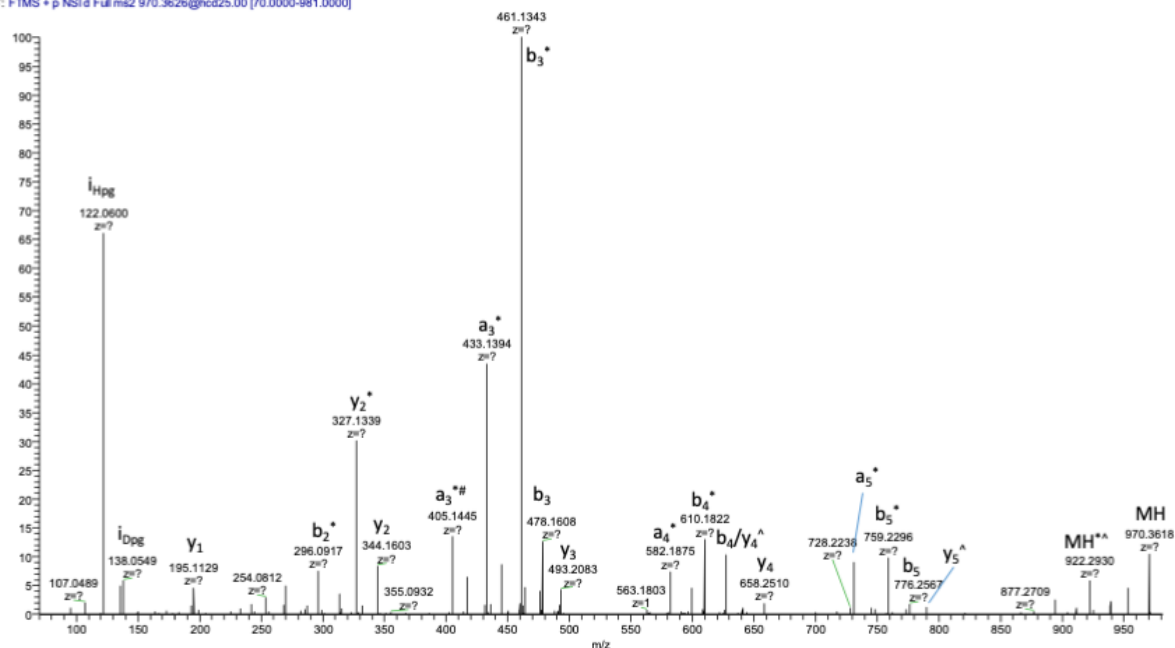




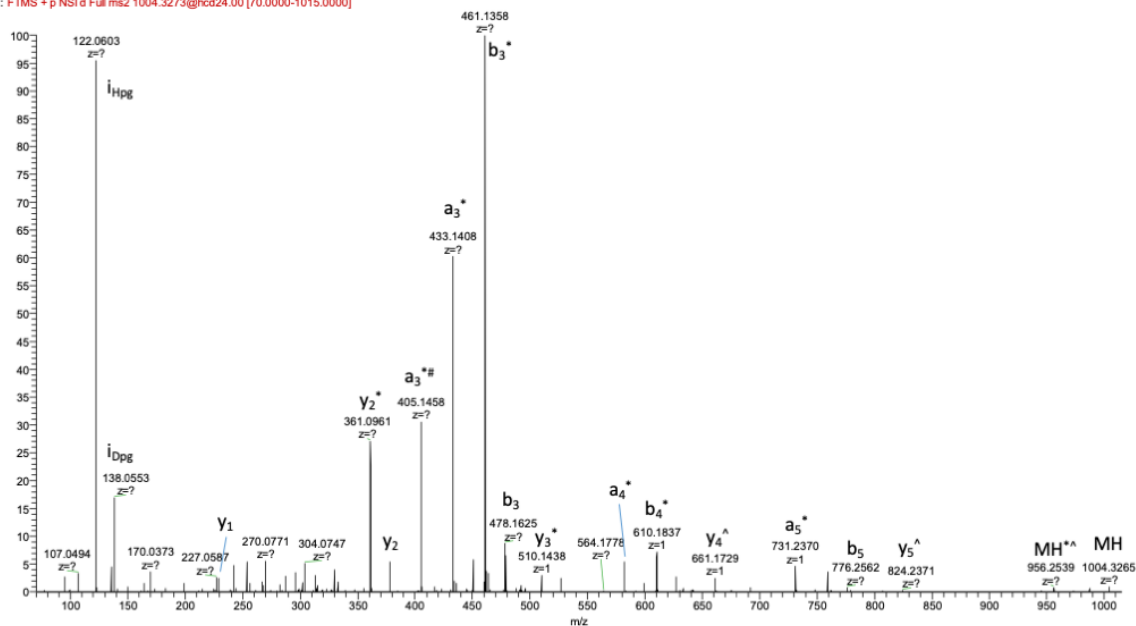
SI Figure S12. Reconstitution of peptide biosynthesis from the teicoplanin NRPS proteins Tcp10 and full length Tcp11 using 3T-loaded M3, ATP, 4-Hpg and Cl-Bht (**4**) as the substrate for module 6 as determined by LCMS analysis (positive mode), with solid lines indicating methylamide peptides (PCP-bound) and dashed lines indicating hydrolysed peptides (tripeptide **3T**: black line; tetrapeptide **4T**: dark grey line; pentapeptide: light grey line **5T**; Cl-Bht containing hexapeptide **6T-4**: green line).



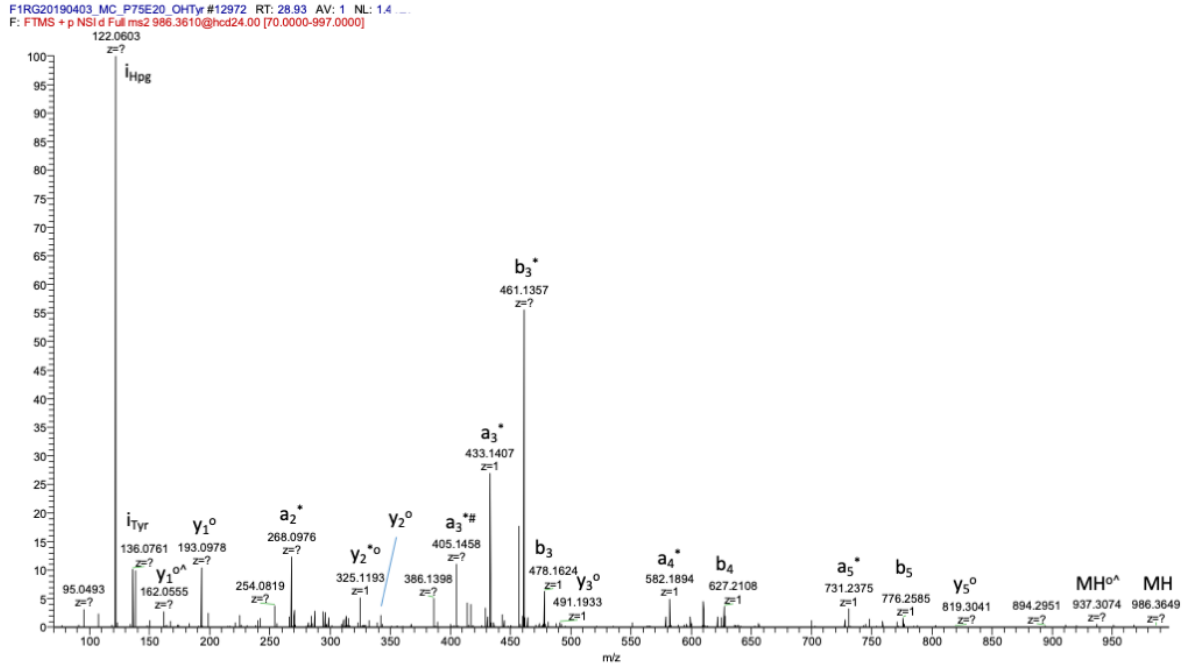
SI Figure S13. Evaluation of hexapeptide yield in pentapeptide **5T** extension experiments with Tyr/Cl-Bht (**1/4**) substrate competition using different Tcp39 concentrations. Black column indicates hexapeptide **6T-4** yield in the control reaction for module 6 using Cl-Bht (**4**) only as a substrate; red column indicates hexapeptide **6T-4** yield using a mixture of substrates (Tyr and Cl-Bht) without Tcp39; blue column indicates hexapeptide **6T-4** yield when substrate competition reactions were supplemented with different amounts of the Tcp39 thioesterase.



SI Figure S14. MS² spectra of Tyr₆ methylamide hexapeptide (**6T-1**) from NRPS peptide assay annotated with major fragments. i = immonium ion, b = b-ion (N-terminal containing), y = y-ion (C-terminal containing), MH = singly-charged parent (intact peptide). Subscript number = number of amino acids, for immonium ions = three letter amino acid code. Superscript symbol = neutral loss, as follows: # = CO, * = NH₃, ^ = methylamine (C-terminal group), ° = H₂O

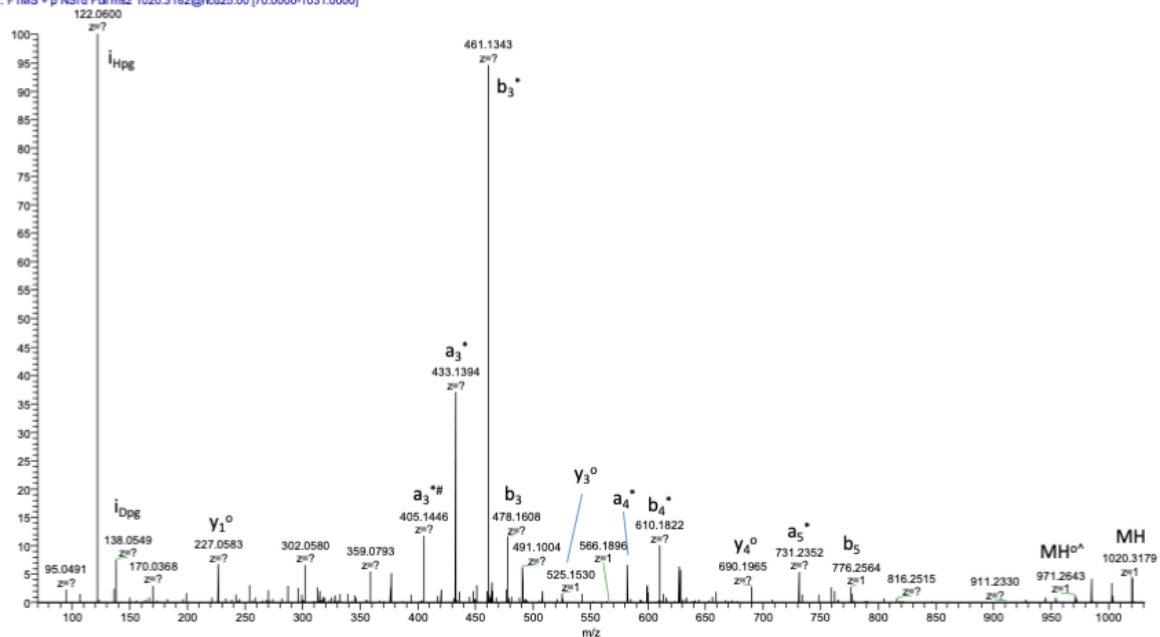


SI Figure S15. MS² spectra of Cl-Tyr₆ methylamide hexapeptide (**6T-2**) from NRPS peptide assay annotated with major fragments. i = immonium ion, b = b-ion (N-terminal containing), y = y-ion (C-terminal containing), MH = singly-charged parent (intact peptide). Subscript number = number of amino acids, for immonium ions = three letter amino acid code. Superscript symbol = neutral loss, as follows: # = CO, * = NH₃, ^ = methylamine (C-terminal group), ° = H₂O



SI Figure S16. MS² spectra of Bht₆ methylamide hexapeptide (**6T-3**) from NRPS peptide assay annotated with major fragments. i = immonium ion, b = b-ion (N-terminal containing), y = y-ion (C-terminal containing), MH = singly-charged parent (intact peptide). Subscript number = number of amino acids, for immonium ions = three letter amino acid code. Superscript symbol = neutral loss, as follows: # = CO, * = NH₃, ^ = methylamine (C-terminal group), ° = H₂O

F1RG20190301_MC_P75E19_C10HTy #15069 RT: 31.07 AV: 1 NL: 1
 T: FTMS + p NSId Full ms2 1020.3182@hcd25.00 [70.0000-1031.0000]



SI Figure S17. MS² spectra of Cl-Bht₆ methylamide (**6T-4**) hexapeptide from NRPS peptide assay annotated with major fragments. i = immonium ion, b = b-ion (N-terminal containing), y = y-ion (C-terminal containing), MH = singly-charged parent (intact peptide). Subscript number = number of amino acids, for immonium ions = three letter amino acid code. Superscript symbol = neutral loss, as follows: # = CO, * = NH₃, ^ = methylamine (C-terminal group), ^o = H₂O

SI Tables

SI Table S1. A domain selectivity code comparison of Tyr-activating A domains from module 6 of different GPAs (including mutant prepared in this study) together with that of module 6 from arylomycin

Protein ^(ref)	A ₆ selectivity code ¹										Description
A ₆ Teicoplanin ²	D	A	S	T	I	A	G	V	C	K	Tyr permissive pocket (tei-type)
A ₆ UK-68597 ³	D	A	S	T	I	A	G	V	C	K	
A ₆ Balhimycin ⁴	D	A	S	T	L	G	A	I	C	K	Tyr permissive pocket (bal-type)
A ₆ Vancomycin ⁵	D	A	S	T	L	G	A	I	C	K	
A ₆ Chloroerymomycin ⁶	D	A	S	T	L	G	A	I	C	K	Tyr specific pocket
A ₆ Arylomycin ⁷	D	A	S	T	V	A	A	V	C	K	
A ₆ A40926 ⁸	D	A	S	T	V	A	A	V	C	K	
A ₆ Complestatin ⁹	D	A	S	T	V	A	A	V	C	K	
A ₆ Kistamicin ¹⁰	D	A	S	T	V	A	A	V	C	K	
A ₆ Kistamicin-mut [*]	D	A	S	T	I	A	G	V	C	K	Tyr permissive pocket

* This study

SI Table S2. Synthetic gene sequences encoding teicoplanin biosynthetic enzymes expressed in this study

Synthetic gene names and sequences (restriction sites are underlined)
<p>Tcp11 module 4 (C-A-PCP-E architecture)</p> <p><u>CCATGGGT</u>ACCCAAGCGCGTATCGAAGATATTTGGCCACTGTCCCGTTACAGGAAGGACTTCTTTTCCACACCGTTTACGATGACGAAGGCCGGACGTATACGTTGGGCACTGGATTCTCGATCTGGATGGCCAGTCGATGCGGGCGCGCTGC GCGCGCATGGGAAGCGTTACTGGCACGCCATGCCGCGTTGCGTGCGTGCTCCGTC AACGCCGACGGGCGAAACG GTACAAGTAGTTGCGGGCCGCGTGGAACTCCCGTGGCGCGTTCGTTGATCTGGCACATCTGGATGATCCGGAACACGC GGTTCTGTAATTAGCCGACGAAGATCGCCTCCGCCATTTGATGTGGCAAACCGCCGCTGTTACGTCTGACCCTGATT CGTCTGGCTGATGACCGTCACCGCTTGGTGGTGACCTGCCATCACGCGGTGATGGATGGCTGGAGCTTGCCATTGTG TTTGACGAACTGACGGCCCTTATGCTGCAGGCGACGGTCCAGCACAGCTGCCGGCCGTCACGCCCTATCGCGAGTAT TTGGCCTGGCTTAGTCGCCAGGATAAGCCAAGTGCCCTTGGCGGCTGGGCCGCGGAATTACGCGGCGCCAGGAACC CACTTTAGTCGCCCCGGCTGATCCGGGACGCGCACCGGGTATGCCGGAATCTGTGCAAGTGCAGTTGTCTCCGGAAC T GACCCGCAGTCTCGCCGAGCTGGCTCGCGGGCGCGGTCTGACGCTCAATACCGT GATCCAAGGAGCGTGGGCGTTGG TTCTGGCGCGCTTAACGGGTCGTACCGACGTGGTGTGGCGCGACAGTTAGCGCACGCCCGGCCATCTTCCGGGTG TCGAGGCGATGGTCGGCTGTTTCTCAACTGTGCCAGTGCGTGTTCGGCTGCGCGGGTCAGCTCCCGTCTGTGAGA TGCTGGCAGAGCTCCAGAAACGCCAATCAGCCTAATTCCGGAACAGTTTCGTGCGTCTGGCGGACATTAGAAAGCGG CGGGTCTGGCGCGGTGTTTCGACACTCTGTTGGTATTCGAAAATTCCCTCGGGAAC TGACGACTCGCGCAGTGCAG ATGCGTTCGGCATTGCGTCCACCAGGGCCGTGAAGCTGCGCATTACCGCTGACACTGGTAGCCGTGCCGGGCGAG TCCATGTTGTTAAGTTGATTACCTGACCGAAGTGTGGACGCCGACAGCGGCCAGTATTCTGAACGCTTTACCC GCGTTCTGCGCGTCTGACCGATGCAGGTGAGCTGACAGTGCCGCGATTGATGTGACGTCGCGTCCGGAACGCGAC CGTGTGGCCCGTGGGGTGTGCAAGTGGCGTCCGGATCGCTGGCGCTGGATTGTTGACGTCAGGTGCGC CCAGCGCCCGGATGAGGTGGCCGTGGCAGACGGCGATCGGGTGTGAGCTTCGGCGAACTTGGCGAACGTGCTGAA CGCCTGGCGGGCCACTGAGTGCGCGTGGCGTGGCGCGTGGTGTGATCGGGTAGCGGTTGTAATGGAGCGTTCTGGCG</p>

Synthetic gene names and sequences (restriction sites are underlined)

AACTCATCGC GACACTGTTAGCGGTTTGGCGCGGGAGCAGCTTTCGTTCCAGTGGACCCGGCCTATCCAGCAGAAC
GTGTGAAGTTCCTGTTGACTGACGCGGAACCGGTAGCCGCACTGTACCGCGGCGTTCGCGCCGAGTACTGGAC
GGAGGACTGGAAGCGATCGTCGTAGATGATCCGGGACGTGGCCCGCGTGGCTCCATGTCTCCCGTTCCGACAGG
TCCAGACGATCTGGCCTATGTTATGTACACCACTGGCAGCACCGGGACTCCGAAAGGTGTTGCTGTGTCGATGGGGA
TGTTGCAGCGCTGGTGGGTGATCCGGGTTGGCGGACGGGTCCAGGTGATACCGTGCTTATGCACGCAAGCCATGCGT
TTGATATCTCCTATTGAGATTTGGGTGCCCTGTAAAGCGGCGCACGTGTCATGATTGCGGGTCCGGGCGCCGTGG
ACGGTGCAGCACTGGCTGCCAAGTTGCGGCGGGGTACCAGCCGACATCTGACCGCGGGCGCGTTCGTTGCTGTGCTG
GCTGAAGAATCGCCGGAGTCCGTGGCAGGGTTCGCTGAAGTCTGACCGCGGCGATGCCGTTCCGCTGGCGGCAGT
AGAGCGTGTCCGTCGGGCGCTGCTGACGTCGCGTGCAGCATCTGTATGGTCCGACCGAAACCACTCTGTGCGCAAC
CTGGTGGCTTCTGGAGCCAGGGGATGAGACAGGGCCGTAAGTCCGATTGGCCGCCACTGGCTGGTGCAGCGTGT
ATGTTTTGGATGCTTTTCTCCGCCGCTCCCGCCGGTACTACGGGCGAATTATACGTGGCCGGCGCTGGTGTGGCC
AAGGTTACCTGGGGCGTCCGGCGCTGACCGCGAACGTTTTGTTGCGGATCCGTTTGGCCCTGGCGGCCGATGTACC
GTACCGGGGATCTGGCGTATTGGACTGAACAAGGCACTCTCGTTTTGCGGGTCTGCCGATGATCAAGTAAAATCC
GTGGCTATCGCGTAGAACCGGGAGAAGTTGAAGCGGTACTGGGAGGGTCCAGGTGTTGCACAGGCCGTGGTTTG
TGTGCGCGGTGAGCATCTGATTGGATATGTCGTCGCGGAAGCCGTCGGGATCTGGACCCAGAACGCTGCGGGCC
GTTTGGCGGCGACCCTGCCGGAGTTTATGGTCCCCGCGCAGTCTGGTGCTGGCCGACCTGCCGCTGACGTTAATG
GGAAAGTGGATCGCCCTGCTTACCAGAACCTGACTTTCAGCGAAATCTACCGGTCGCGCCCGCAACGGCTGCAG
AACGTATCCTGTGCGACGTGTTTGGGGCGTGTGGCCCTGACCGCGTAGGCGTTGAGGATTCTTCTTTCGCTTAG
GCGGGGATTCAATCAGCTCGATGCAGGTCGCGGCACGCGCTCGCCGTGAGGGCATCGTTTAAACCCGCGCAAGTC
TTTGAACACCGTACTCCAGAACGTCGCGCGGTTGGCCCGCGCGGGTTCAGCACGCCCTGATCGCAGTGCAGC
GGATGCAGGGTGGGCGAGATCCCTGGACCCCTGTCATGCGCGCTCTTGGTGATGATGCCGTGCGTCCGGGCTTCGC
GCAAGCGCGTGTGGTAGTCGCGCCGCGGCTGACCCCGATGCGCTGACGGGCGCGCTCCGTGACGTCCTGGATA
CGCATGACGTTCTTCGTGCGCGTGTGAACCGGATCGTCGTCGATCGTCCAGAACGTTGGTGCAGTACCGGCCGCTG
ATCTGTGACCCGCGTGTGTTGATTCTGACGGTATTGATGCCCCGTCAGAACCGGAAGCTGCGACGGCGGCTGGCA
CGCTGGATCCTTCAGCAGGGATCATGCTTCGCGCGGTCTGGCTGGATGCGGGCGATGCCGAACCGGGTTCGCTGGCT
CTGGTCGCGCACCATCTGGTGGTCGACGCACTGAGTGGTCGATCTTGTCCGGACCTGCAGGCAGCATAACAGGAA
GTACTGGCCGGTGCAGCCCTGCCCTTGAACCTGCTGCGACATCTTATCGCAATGGGCGCGCCGTTGACCGAACAG
GCTAGCTACCGTCCACGTTAGCAGAACTGGACCACTGGGTGACCGTGTGGATGCCGCGGAGCCGCTCTGGCAGA
ACATCACGGCCAGGCACATAGCTGGAGCGCCACTTAAAGCGGTGCCGTTGCTGGACATCTGGTATCCCGCATGCCGG
TGCGTTTCATTGCGGCATTAGGAGGTTCTGTTAGCAGGCTGGCCGCGCGGTGGCGCGCTGGCGCGGTGATGACG
CCGGCGTTTTGGTGGACGTTGAAGGACACGGTCCGGCACGCTGCGGATGGGGAGGACCTGCTTCGTACGGTGGGGTG
GTTACCTCGGTACACCCAGTTCGCTCGATGTATCGGGAGTGGACTTGGCGGCCGAGCTGCCGGTGTGCGGCTGC
AGGTGAACTCCTGAAAAGCGTGAAGATCAGGTGCGTGGCCCGCGGAGATGGCTTTGGCTTCGGTTTATTACGTCA
TCTGAATCCTGATACCGCTGAGCGCTTAGCCGCCCTGCCGGCACCGCAGATTGGCTTCAACTATCTTGGCCGAGCGG
CGTCGCGGCTGAAGCCGTACCCTGGCAGGTGCGCGGCGGTTGCTCGGAGCAGGAGAAGCAGGTCCCAGCTGGTC
CTCGCGCATCCCCTGGAGGCTGGCGCAGATGTTGCTGATACTCCTGATGGTCCACTTTTGGCTTACCCTGGACGGTC
GCGACCTGGCTCCGGTGCAGTTGAGCTTCTGGGCGAGGCGTGGTTGGAGCTCCTGACGGGCGCTGGTACTCATGCG
GGCGACCTCGTGCGGGTGGCCATACGCCGGCTGATTTGCACTTGGTGAAGTAACGCAGCGGGATGTACAGCCTT
AGAAGCAGCGGCCGAGAGTTTGGTGGCGGGTTAGAACTCGAG

Tcp11 module 5 (C-A-PCP-E architecture)

CCATGGGTACGACCCCGACTACGTCCCCTGGTTCAGCAGGCCTGACGGATATCTGGCCGCTGAGCCCCTACAGCGTG
GCATGTTATTCGAACGGGCCCTCGATGAGGATGGCGTTGATGTGTACCAGTCTCAGCGTATTCTCGATCTGGACGGTC
CTCTGGACGCAGACCGGCTCCGTGCAGCGTGGCGCACTCTGGTTGCCGTCATGAGTCACTCCGCACTAGCTTCCACC
AGCTGGAAAGCGGGCGAAACAGTCCAAGTTGTCGTGGATCAGGCCGATATTGGTTGGCGTGTGGCGGATGTTAGCCAT
CGCGCAGAAGCAGATGCAGTGCAGAAGTGGGGCGGCTGTTGGCCGAAGATCAGGCGCAGCGCTTTGACGTTACGC
GCGCCCCACTGCTGCGCTTGTGCTCGTTCGCTCGGCGCCGATCGTCATCGCCTGGTCTGTCACCTCTACCACATTGTG
CTGGACGGCTGGTCTACCCCTATCATCTTGGGCGAAATGCCGACGATGCAGGTGCTCCGAGCACCGCTACCACA
CCCAGCCCAGTGATTACCTGGCCTGGTTAAACCGTCAAGATGATGCCGCGACCCGCGTGCATGGCGTAGCGAATTG
GCCGGCTGTGATGAGCCGACTTGTAGTGGACGCCGACGCGGATAAGACTATGGTTATGCCGGGCGAGCACTGCGAAG
GCTTGTGCGAACAAGCGACTCGCCGCTTAAACCGAATTTGCTCGGGTTCATGCTGTTACCCTGTCAACGATCTTACAGG
GAGCATGGGCACCTGTAATTGCTGCTTGGCCGGCTACAGACGTGGTGTTCGGGACAGTGGTTTCTGGCCGCCCGG
CGGAAGTCCCAGCGTTGATCGATGGTGGGTATGTTTAAACACCGTCCCCTCCGCGTTCGCTGGATGGTGGGC
GTCCCCTGCTGGACCTCCTGCATGACCTTCAGGCTCGCAGTCAGCACTTACGGAACCAACACTTAGGGTGCCTG
ATATTCAACGCGCGCAGGGCCGGTGCAGTGTGATACGATCATGATGGTAGAAAACCTACCAATGGATGCTGCCG
GTTTGGGCGGCGAGGGCGGTGTTGATCGGTTAGTTCGACCCGACCCGACCGCTATCCGCTGACCATGTCAG

Synthetic gene names and sequences (restriction sites are underlined)

CGTCGCCAGGCGAGCGCCTTCAGATCCGCGTCAGCTACCGTCCGGATCGCATCGAACGCGAAACCGCAGCCGAGGCA
GCGGGCCAAGTAGTACGTGTTATTGAACGCATTGTCGCTGAGCCGCTTTGGCGGTCGGTCGCTCTGGGAGTCAACGGT
GAGTCCGTGCGCGAACGTGTGGTTGAACGCTGGAATGCCACAGGCGTGGCCGAGGAGGGAGCAGCGTATTGACAG
GTTTTCGCCGTCGGGCGCTAGCAGTCCCAGCCTGTGGCAGTATCGGGTGGCGGCCGACCCTGTCTTATGCCGCGC
TGGACCGCGAAAGCGATCGCCTGGCAGGGCATCTGGCCGGCCGTGGAGTGCGCCGCGGCCACCGTGTGGTGTGGT
GATGGAACGCGGTACGGATCTGTTCTGCTCCTTGCTATTGGTAAAGCGGGTGCCGCGTATGTTCTGTGAACTT
GGATTACCCGCGTGACCGCATTGAACGTATGCTGACCGATGCGGGCGTTAGTGTGGCCGTATGTGCCAGGCAACCTC
GGGTGCGGTTCCGGATGGGCTGGCGCCGGTGGTAATGGACTCGCCAGCAATTGCGGCCGCCCTTCGGAAGCGCCGC
CAATTACGGTTCGGGCGCATGACCTGGCGTACGTTATGTACACGTCGGTTCGACCGGCGTGCCTAAAGCGTTGCCG
TTCCGCACGGTTCGGTTCGCCCTTGCCGGTATCCGGGCTGGTCCGTGGGTCCGGGCGATGGAGTCTTAATGCATG
CGCCGCACGCTTTGACGCTCATTATTGAAATCTGGGTACCGTTCGTTTCGGGAGCGCATGTGGTGGTAGCGGACC
CCGGCGCGGTGGATGCACACGCTGCGGGAAGCCATTGATCGCGGTGTACCACCGTACATCTGACGGCGGGGAGT
TTCCGGTTTTGGCGAGGAAAGCCGGATGCGTTTTGCGGACTTCGCGAGGTGCTGACCGGTGGTACGCGATTCC
GTTGGCGAGTGTGTGCGTTACGCGAAACATGCCGGAAATTCGCGTGCGCCACTGTATGGCCCGACCGAGACAA
CTCTGTGCGCCACTTGGCATCTGATCGAGCCTGGCGTTGCGACAGGTGATACGTTACCAATTGGACGTCCGCTGGCGG
GGCGTCGTGCGTATGTAAGTGGATGCCTTCTGACGCTGTGGCTCCGAATGTAACCGGTGAACTGTACCTGGCCGGGG
CGGGCCTGGCGCGCGTTATCTTGGCGCTGCGGCCACCGCCGAACGTTTTGTCGCCGATCCCTTGTGCGGGC
GAACGTATGTATCGTACTGGTATCTGGCTCGTTGGACGGAACAAGGGGAACTGCTGTTGACGGCCGTGCCGATGC
GCAGGTCAAGATTCGTGGCTACCGCGTCAACCGCTGAAATCGAAGCTGCGCTGACGGCGATTCCGGAAGTGCAC
AAGCAGTTGTGGTAGCCCGTGAAGATGGCCCGGAGAGAAACGTTAATTGCTTATGTTACCGCCGCTGCCAACCG
GGCCAGATCCGGCAGCCGTACGCGAACATCTGGGTGAGCGCCTGCCGGAGTTTATGGTACCAGCGGCAGTGGTCT
TTAGACGGCTTTCCTCTGACCATTAACGGGAAAATCGATCGCGCCGCGTCCAGCACCGGAACGCTCCGGCAAACC
GGCGGGACGGGAACCGCGTACGGAAGTTAACGGGTTCTGTGCGACTTATTCGCGGATCTGCTGGGCTGGATCGTG
TCGGCGCCGATGACTCGTTCCTTGTGCTTGGCGGGATAGTATCGTGAGTATGCAGCTGGCGGCCGCGCGCGCCG
GAAGGGGTAGTGTGGCGCAAAAGATGTGTTGCAACAGAAAACGCCGCGCAGCGATTGCGGCTGTCCCGTCCGCG
GAGGCCAACGCCAGCGGGCCAGACGATGGGGTAGGTGAAGTGCCTGGACGCCAGTACGCGTGGTCTGTTAGA
TCGCGACCCGGGAACGATGAATCGTTCGCAATGGCGCAGTGGGTAACAGTGGGTGCACCTGATACCCTGTCCCGTG
ATGTGCTGGTGCCTGGTGTGGTGGTCTTACGCGCATGACATGCTCCGTAGCCGTGTAGTGCGGCGGGTACTG
AGGAACCGCGCTTAGTGGTGGCGCCCCGTGGTGCCTGACGCGCGGGTCTGGTGGATCGCGTGGAAAGCCGGGGC
AGGTGAGGTGGATGACCTGACCGAACGTAGTGTGCTGCAAGCTGCGACACGCTTAGATCCAGGTGCCGGTGTATGG
TACGTGCAGTGTGGTTGATGCTGGGCCAGTGCCTGGGACGTTTATGGTGTAGTCAACACCTGGTCTGTGACG
CTGTCTCATGGCGTATTCTGCTGCCAGATCTGCGCGCTGCCTGTGAGGCTGTGGCGGGGGCGGAGCCGGCCCTC
GATCCGGTGGATGTGAGCTTTCGCCGTTGGGCCGTACCTTAACCGATCAGGCAGCCACCCGCACTGCGGAACTTGGC
ACGTGGACTCGCATCCTGGGTGGAGCCCAACCCGCTTCGGCGCACTGGACCCGGGACGGGACACTATCAGTACGGC
TGGCCGTGCGACCTGGACCGTCCCGCAGGATCGCGCAGGTGTGCTGGTGAACGTGCACTTCCGCGTTTACTGTGG
CGTGCATGAAGTCTGTTGGCGACTGGCCGGTGCAGTTGCTCGTGGCGGGCGGCGCAGCGGTGGTAGTCGACG
TTGAAGGTATGGTCCCGCCGCTTGGGGAGATGGACCTGAGTGCACCGTCCGTTGGTTTACGGATGTGCATCCTC
TGCGCCTGGACGTTACGGGGATTGACACCGCGGAAGTCAATGCGGGTGGTGGCGCGGGCCGGCTCTCAAACC
GTGAAGGAGAATGTGCGTGCAGTACCCGATGGCGGTTTGGGGTATGGGATGCTGCGCCATCTGAATGCTGAGACTGG
CCGGTACTCGCAGCGCAGCCTGCGGCCGAAATGGTTTTCAACTATCTTGGTCCGTTTTCTGAAGGCCGGGGCGGCGA
TGTGCAGCCGTGGCAACAGCTCGGAACCATGGCGGCACGGCCGAGCCGGACATGCCGCTGCGTATGCCGTGGAAA
TCGACGCGGCTGTGCTGGATGCAGCCGACGGCCCTCGCCTGAATTGACCGTGCATGGGCCGGCCGCTGTTGGA
GCTGCGGAAGCCGAATCGCTCGGCCGCGCTGGCAGGATGTGTTGGCCGGACTGGCCGTCCACGCCGGCGATGTCCG
CGCAGGCGGCCATACCCCAAGCGATTTCCCGCTGACGGCTGTCTGGCCAGGAGGTGGCTCAGATCGAGGCAGAGT
TCGATGACGACCTCGAG

Tcp11 module 6 (C-A-PCP architecture)

CCATGGGTACGCAAGCACGGATTGAACTGCTGGATATTTGGCCGCTGAGTCCACTGCAGGAAGGCCTGTTATTTTCATG
CCGTTGATGAACGTGGGCCAGATGTCTACGCTAGCATGCGTACCCTGGCAGTTGATGGTCCGCTGGACGTGCCCCGGT
TTCGGGCTTCTGGCAGGCGGTTTTAGATCGCCATGCAGCACTCCGCGCGAGCTTCCACCAACTGGAGAGTGGCGCTG
CGGTGCAAGCCATTGCCCGTGAAGTTACCCTCCCGTGGCAAGAACTGATTTATCGGATCTCCCCGAGGACGTAGCGT
TGGCCGAGTTTGACCGCTGGCGGCGCAGCTGCGTACGAAACGCTTTGACCTGACCCGTGCGCCGCTTACGCTTC
ATTTGGTTCGCTTAGGCGAACCGCCCATCGCTTGCATTTGCAAGCCACCACATTTTCATGTGATGGCTGGTCACTGCC
TATCGTAGTACCGAAGTCATGGCGGCCATGAAGGTCGTGGCCTGCCGGCGCCGACATCCTATCGCGACTATCTGGT
GTGGCTTGGCCGAAGACAAAGAAGCAGCCCGTCAGGCTTGGCGTGGTGAATTGGCTGGCTCGATGAAGCGACCC
ATGTGGTTCGCTGACACCATCACCGCGCAATTGCGCCACAACGCGTCCGCTTGGACTTGACGATGAGCTTAGCC

Synthetic gene names and sequences (restriction sites are underlined)

GTCGTCTGGTCCGCTTTACCCGTCGTCACGGGGTGACAATGAACACACTGTTCCAAGGCATTTGGTCGCTGCTGTTAGC
CCGTTAACTGGTCGCGATGACGTTGTGTTCCGGTCAGCGGTTGCAGGTCGCCGGCTGAGATCCCGGGCGTTGGTTC
TGTTGTGGGGTTGTTTCATGAATACTCTGCCGGTACGTGTTGCGCTGGATGGCGCGGAGCCATTTCTGGACATGCTCAC
GGATTTGCAGCGTCGTCAGGTAGCTATGATGGCGCATCAGCACTTAGGCTGTGAGAAATCAAGCAGGTAGCGGGCC
CGGGTGCAGCATTGACACGCTTGTGGTCTTTGAAAATATCCTCGGCCACCTCGCCCTAGCGACGATCCGGATGCCGT
GGTATGCGTCCCGCTGAAATCCGGAAGATAGCGGCCATTATCCCGTGTCTATGCGCGCGTCGGAAGATGGTGGTG
TTCATGGTGAATTTATCTTTCCGCCGATGCCTTCGACCGGGCGGAAGCTGAAGGAATGCTGGCCGCCATTATTGAGG
CGCTGCGGCAGGTGGTGGCAGAACCAGGTATTGCGGTTGGCCGTATCGGACTGGTAGGCGCGGCAGAACGCCGCCA
AGTGGTCCGCGAATGGAACGAAACAGATGCGCCGCTCACGGCACAACCGCTTCCCATGTTGTTCCGTCGTCAGCTCA
GCGTTCGCCGGATGCGGTGCGAGTCCGCGATGCAGCACGAGTCTGTATTGAGGCCCTGTTAGGTGAAGCGGAAG
GCCTTGCGCGTCGCTTGTGCGAAGTGGCGTGCGCCGTGAAACTCGCGTAGCAGTCTGGTCGAGCGGAGCGCTGAG
CTGGTAGTGGCCCTGCTGGGCGTAAGCCTGGCCGGTGGCGTTTTCTGTCGCCGGTGGATCCGGATTATCCGGGCGATCG
CATCGCTCTGATGCTCGCAGATGCGGCTCCGAGGTCCTGGTGTGCACGGCACGTACCCGCTCTGTCGTGCCTGGCGA
TTTTGCCGGCGCCGTGCTTGTCTGGACGAACCGAGCGCTGCCGGACCGCAGGTGAGCCTTCCACGCGTTGCCGCGG
CGATGGTGCGTACGTAATCTACACCTCCGGCTCGACGGGTGTACCGAAAGCGTTCTGGTGACCCATGCCGGTCTGGG
TAATCTGGCTTCGCGCAGATTGAGCGCTTTGGTGTGACCTCGTCCAGTCGTGTTCTCCAATTTGCAGCTCTCGGCTTT
GACGCGCGGATCAGCGAGGTTTGCATGGCGCTGTTGTCTGGCGGATCCATTGTTCTGGCGGATGCCGAACACATGCC
ACCTCGCTTTCTTAGGTGACGCCGTCGCTCGTGGACATTACGCATGTGACGGTCCCGCCGTCCTGTTAGCAGTT
GAGGATGACCTGCCGGATGATCTCACACTGATTGTTGCAGGGGAAGCCTGTCTCCGGCTCTGGTGGATCGCTGGAGT
CCGGGCCCGCTATGATCAATGGCTATGGACCGACCGAGACTACTGTCTGTGCAACCATGAGCTCTCTCTGTACCG
GGCCGTGGAGCAGTGCCAATTGGCGGCCAATCGCGAACATGCGCACCTACGTGCTGGACGCGTTCTCCAGCCAGT
GCCACCAGGGATACCGGTGAATTATACGTACCCGGCGTGGGTCTGGCACGCGGATACTTGGGTGCTCCCGGTCTGA
CCGCGGAACGCTTCGTAGCGGACCCGTTAGCCCGGGCGGTGCGATGTATCGCACTGGCGATCGCGCGCGTTGGACG
GGCGATGGTGAAGTGGTTTTGCGGGGCGCGTTGATGCTCAGGTCAAAGTGCCTGGGTATCGCATTGAACCCGGGA
GATTGAAGCCGTGTTGGCAGAACATCCCGGCGTCGCGCAGGTTGTAGTAGTCGCGCGTCAAGACGGTCCGGGTGAGA
AACAGCTGGTGGCTACGTAGTACCTGCCCGGTCCTACCGCCGAGGCGAGCACTCTGATCTCGGCGTTACGCGAAG
CTGCAGCAGCCCGCTGCCGGAGCACATGGTACCGCGGCCCTTTGTGCTTTGGACGCCATGCCGCTTACGCCAATG
GGAAAGTTGATCACCGTGCTTACAGGCGCCGATTTCGCCGGGATGTCTGCAGGGCGTGATCCTCGGACAGCGTTG
GAGGCCAAACTGTGCGAACTGTTTGCCGAAGTGTGGGTTTGGAGCAGGTTGGAGCCGATGATTCTTTCTTGAAGT
GGCGGGGACAGCATCACGAGTATGCAGCTGTCCACGCGCGCTGCCCGGAAGGGTTGGAAGTACCCCGTGGCAGG
TGTTGACGAGAAAAACCCGGAACGTCTGGCGGCGTTAGTCAAGGAGATTCCAGCTGATGGCGGCGCAACGGGCGC
GCCGGAACCGGCGGCTGGTACCTTGGTGCCTGTCTCCGAACAGATGGATCAGCTGGAAGCGGGTCCGGCCGGTG
AACTCGAG

Tcp39

CCATGGCGATGCCTGTTCCGTGGTTCTCGGCACCACGTCCGTTAGCGGCGCCACGTTTACGCCTGGTGTGCTTTCCGTA
TGCTGGTGGGAACGCAGCTACCTACCGCAGTTGGGCGGGACTGCTGCCTCCTGGTGTGAGTTAGTCGCGGGCGCTCCT
GCCGGGTCGTGCGAAGCGTTAGACGAACCACCGTGGTGCACCTCGACGTGTTGCTGAGCGAACTGGTGGCCGCGG
CTGGTCCGCTGTTGGGCCCGGTTCCGCTGATCCTGTTTGGCCATTCCCTTGGGGCCACCGTAGCGTACGAGTTTGGTGC
CGCCTTGGCGACGGAACATGGGTGTGTGCCCGCAGCGCTTCTGGTTTCGGGTGCGCCCCGACCAACCGTTCCGCCCGG
CCGTAAGTTCGCGGACTTAGCGATGACGATCTGGAACGCATCTTGGGCAAACGCACCGATCTGCCGGCAGGATGGC
TGACGGCAGAGCTCAAACCGTTCGTTTCCGGCTCTGCGTGCCGATCTGCAGCTCCTGGACGGCTATCGCTGGCGTCC
TGGCCCTTACCGGGCGTTCCGGTAGTGGCCTTTGGCGGTGATGCGGATCCAGATGTGTCTGCGGCTGATCTGCGTGC
CTGGCAACGCTGCACAACTGGCCCCGCTGTACCCACGTAAGTGGCAGGCGATCACTTCTTATTGCCCATCAGCCGTTT
CGGGAAGTCTTCCGCCACGGTGGGCGAATTCGCGGCGTACCGATTGCCCGCCTCGAG

SI Table S3. Primer sequences and template DNA used for cloning of Tcp11 modules with altered architecture, Tcp11 4-5 di-module and full length Tcp11

Final construct	Primers	Insert template DNA	Vector template DNA
Module 4 C-A-PCP-E-C	M4CATEC-fwd AGAAGGAGATATACCATGAAACATCACCAT M4CATEC-rev CCAAGCACTCTCGAGTCTGCGGCCACGCCTGT	pET-GB1-1d, encoding Tcp11 di-module 45 protein (C-A-PCP-E-C-A- PCP-E architecture)	-
	Vector-M4CATEC-fwd CTCGAGAGTGCTTGGAGTCATCC Vector-M4CATEC-rev GGTATATCTCCTTCTTAAAGTTAAACAAAATTATTCTAGA GGG	-	pET-GB1-1d series
Module 5 A-PCP-E-C	M5ATEC-Fragment1.fwd AATCTTTATTTTCAGGGGAGCAGCGTATTGACAGG M5ATEC-Fragment1.rev AGGTCTGGTACTGCTGCCTCGATCTGAG	pET-GB1-1d, encoding Tcp11 module 5 (C-A-PCP-E architecture)	-
	M56Fragment 2.fwd AGCAGTACCAGACCTGCTGGATATTTGG M56Fragment 2.rev CCAAGCACTCTCGAGCGTGAGCGGCGCATC	pET-GB1-1d, encoding Tcp11 module 6 (C-A-PCP architecture)	-
	Vector-M5ATEC.fwd CTCGAGAGTGCTTGGAGTCATCC Vector-M5ATEC.rev CTGAAAATAAAGATTCTCAGAACCACTGCCA	-	pET-GB1-1d series
	M6-AT.fwd AATCTTTATTTTCAGGCACAACCGCTTCCCAT M6-AT.rev GCAGCCGGATCAAGCTTACTTCTCGAA	pET-GB1-1d, encoding Tcp11 module 6 (C-A-PCP architecture)	-
Module 6 A-PCP	Vector-M6.fwd GCTTGATCCGGCTGCTAACAAAG Vector-M6.rev CTGAAAATAAAGATTCTCAGAACCACTGCCA	-	pET-GB1-1d series
	45Fr_1-rev TCAGGCCTGGGGCCGCTGCTTCTAAGG 45Fr_1-fwd AGAAGGAGATATACCATGAAACATCACCAT 45Fr_2-rev GCAGCCGGATCAAGCTTACTTCTCGAA	pET-GB1-1d, encoding Tcp11 module 4 (C-A-PCP-E architecture)	-
Tcp11 di- module 4-5 (C-A-PCP-E- C-A-PCP-E)	45Fr_1-fwd AGAAGGAGATATACCATGAAACATCACCAT 45Fr_2-rev GCAGCCGGATCAAGCTTACTTCTCGAA	pET-GB1-1d, encoding Tcp11 module 5 (C-A-PCP-E architecture)	-
	45Fr_1-fwd GGCCCCAGGCCTGACGGATATCTGGCC		

Final construct	Primers	Insert template DNA	Vector template DNA
	Vector-45-rev GGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTCTAGAGGG Vector-45-fwd GCTTGATCCGGCTGCTAACAAAG	-	pET-GB1-1d series
Full length Tcp11 (C-A-PCP-E-C-A-PCP-E-C-A-PCP)	Fragm_4CATEC.FOR CTTTAAGAAGGAGATATACCATGAAACATCACCAT Fragm_4CATEC.REV CGTGCTCCCTCCTGCGGCCACGCC	pET-GB1-1d, encoding Tcp11 module 4 (C-A-PCP-E-C architecture)	-
	Fragm5-A TEC.FOR GGCCGCAGGAGGGGAGCAGCGTATTGACAGG Fragm_5A TEC_REV GCGGTTGTGCCGTGAGCGGCGCATCTGTTTCG	pET-GB1-1d, encoding Tcp11 module 5 (A-PCP-E-C architecture)	-
	Fragm_6AT.FOR GCCGCTCACGGCACAACCGCTTCCCAT Fragm_6AT.REV TGTTAGCAGCCGGATCAAGCTTACTTCTCGAA	pET-GB1-1d, encoding Tcp11 module 6 (A-PCP architecture)	-
	Vector_Tcp11.REV GGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTCTAGA GGG Vector Tcp11.FOR GCTTGATCCGGCTGCTAACAAAG	-	pET-GB1-1d series

SI Table S4. Synthetic gene sequences of BpsB module 6 (A-PCP), balhimycin MbtH protein and Bhp, as well as primer sequences and template DNA used for cloning of wild type BpsB module 6 and MbtH-like protein

Synthetic gene names and sequences (restriction sites are underlined)
<p>BspB module 6 (A-PCP architecture)</p> <p>GTCGAATCTGCGGTTGGTCTGTTTCATGAATATGTTACCGGTACGTGCTCGTTTGACCGGCGCCGAACCTGTAGTTGATA TGCTGAAAGATCTGCAGGAGCGCCAGGTGGCGATGATGGCGCACCAACACATTGGACTCCCAGAAATTAACAACCTG ACGGGGCCCCGGCGCTGCCTTTGATACCATCGTGGTATTTGAAAATATCCGCCGGCGCCGCTCGTAGCGATGATCCG GATGCCTTGGTGATCCGCCCTGTGGGAATTCAAACGATACCGGTCATTACCCGCTGTCTATGCGTGCGAGTGTCGCA GCCGGTCTGTGCGCGGAGAATTCATTATCGCCCTGACGTGTTGACCGCACCGAAGCCGGCGAGATGGTAGCAGC GATCCTCCGCGCGCTGGAACAGGTGGTGCAGAACCTTGACCCAGTTGGGCAGGTTGGTCTGATTGGCCCGGAAC AGCGCCGCTGGTTGTCGATGAGTGAATCGCACGGATGTTCCGTTAGCGGCTGAAACCTGCCTGTTCTGTTCCGTC GCCAGGCTGAACGCAGCCGGATGCTGTGGCTGTTGAGGACGGTGACACGAGTCTGACATTCCGGCGGTCTGTTAGGG GAGGTGGAGGCACTTGACGCTGCTTGTGGGCGCCGGGTGCGCCGGAACATCGTGTGGGCGTGTGGTGAAC GCAGCGCTGAACTGGCGTTACCATGATGGCGTGAGCTTCGCGGGAGGTGATTTGTACCCGTTGACCCGGACTACC CACGCGAACGCGTTGAATTTATGCTGGCGAATAGCGCGCCGGGGTTATGGTTTGTACTAAAACCCCGTGCAGCG GTTCTGCGGAGTTCGCGGGTACTGTTCTGGTGTGGACGAGTTACCGCAGCCGACCCGGATGTGGAACCTCCACCG GTTGCCCGGAGGACGCGGCGTATGTCATTTACACTTCTGGTCCACTGGCGTACCCAAAGGCGTACTCGTCACGCATT CCGGCCTGGCAACCTGGGGTATGCTCACATTGAACGTATGGCCGTAACCTCGTCGTCACGCGTGTGTCAGCTGAGTG CTACTGGCTTTGATGCTATTGTTAGCGAAGTGTATATGGCCCTGCTGGCTGGCGTACCCTGGTGTCTCCAGATGCTGC CTCCATGCCACCGCGGGTGACCCTGGGTGAAGCATCCGCCGGGCGGGAATTACGCACCTGACGGTAAGTCCATCCG TTCTCGCCAGCGAGGACGACCTTCCGACACTCTTCGTACCGTCTTAACAGGTGGTGAGGCGCTGCCGCCGGCCCTTG TAGACCGCTGGTGCCTGGCCGTCGTGTGATTACGGCCTACGGCCGACAGAACTACCATTGCTCCACCATGTCCCG</p>

Synthetic gene names and sequences (restriction sites are underlined)			
<p>GCCCCTGAGCCCTGGACATGATCAGGTTCCACTGGGTGGTCCGATCCATAACGTGCGTCATTACGTTCTGGATGCTTTC TTGACGCCGGTGCCGCCGGGCGTCTAGGTGAAGTGTATATTACCGGTGTAGGACTCGCGCGCGGCTATCTTGACG CCCCGGATTAACCGCAGAACGCTTTGTGGCGTCCCCGTTTGCGCCGGTGAACGCATGTATCGCTCAGGTGATTTATTC CGCTGGACCCGTGAAGGCCAGTTACTGTTTCGCGGGGCGTGTGATGCCAGGTGAAGGTTCTGGATACCGTGTGGA GCCGGCCGAGATCGAGGCAGTTCTGCCAACACCCGTGGGTGGGCAAGTAGCGGTAAGCGTGCCTCGCGACGGC CCGGGGGACAAACAGTTGGTCGCGTACGTGGTCCCTAGCGCGGATGCGGCTGCCGAAAACGGTACCCTTGCTCTGC ACTGCGGAGTTGGCGGCAGAGCGCTGCCAGAATATATGATGCCAGCTGCGTTTCCTTCCCTGGAACAGATGCCACT GACGCCTAATGGTAAACTGGATCATCTGCGCTGCAGGCACCGATTTTGTGGCATGTCTAGTAAACGCGCACCGCG GACTCCAATGGAGGCACGCTGTGCGCGCTGTTGCCGACGTTCTGGTTAGATCAAGTTGGCCCGGATGACTCCTTC TTCGAATTAGGTGGCGACTCTATTACCTCTATGCAGCTCAGCGCTCGCGCGCTCCGACTGTTTTAGAACTACCCCGT GGCAAGTGTGATGAAAAGACGCCGGAGCGCCTCGCCGTAATTGTTCAAGAACTGGCTGCCGAAGGTGGTACCACG CCGGCTCCGAAACCGGTGAAGGGACGTTAGTGGCACTGAGTCCGGATCAGATGGACTTATTAGAGGCGGGCCTGGC GGGTGAACTCGAG</p>			
<p>Balhimycin MbtH-like protein <u>CATATGTCGAATCCGTTTCGACAACGAAGATGGCAGCTTCTTTGTGCTGGTGAACGATGAAGGGCAACACTCTCTGTGG</u> CCAACGTTTGCAGGAACTTCTGCCGTTGGACTCGGTACATGGCGAAGCTGGTCGTCAGGAATGCTTGGCGTATGTC GAGGAGAATTGGACCGATTACGCCCGAAATCCCTGATTCGCGAAGCAAGTGCCTAACTCGAG</p>			
<p>Bhp <u>CATATGCTGATGACCACCGAACATGGTATTCTGCTGAGCTATCATGATCAGGGTCGTGGTGCACCGTTCTGCTGCTG</u> ACCGGTACAGGTGCACCGAGCAGCGTTTGGGATCTGCATCAGGTTCCGGCTCTGCGTGCAGCAGGTTTTCTGTTATT ACCATGGATAATCGTGGTATTCTCCGAGTGTATGATGGTGCAGATGGTTTTACCGTTGATGATCTGGTTCAGATGTTG CAGCACTGCTGGATCACCTGGATGCAAGCCCGTGTGCTGTTGTTGGCACCAGCATGGGTAGCTATATTGCACAAGAAC TGGCACTGGCACGTCCGGAACCTGGTTGATGCAGTTGTTCTGATGGCAGCATGTGGTCGTAGCAGCCTGGTTCAGCGTG TTCTGGCAGAAGCAGAAGCAGATCTGATTGGTCGTGGCACCGAACCTCCCGGTTATCGTGCAGCAGTTCGTGCAA TGCATAATCTGGGTCCGGCAACCCTGGCAGATGATGATCTGGCAGCAGATTGGCTGGACCTGTTTGCAGCAAGCGAA AATTGGGGTCCGGGTGTTCTGTCACAGCTGCTGCTGAGCGCACTGCCGGATCGTCTGGAAGCATATCGTGCATTTAA GTTCCGTGTCATGTGGTTAGCTTTGAACATGATCTGGTGGCACCTCCGAGCGCAGGTCAAGAACTGGCTGCAGTTATT CCGGGTGCAACCATCGTACCATTCCGGGTGTGGTCAATTTGGTTATCTGGAAAAACCGGAAGCCGTTAATCGTGAA CTGCTGCGTTTTCTGCGTACCGAAAGCGGTGTTGCAGTTACCAGCGGTGCAAGTCCGCGTACACCGGAAGAACTGTAA AAGCTT</p>			
Final construct	Primers	Insert template DNA	Vector template DNA
BpsB module 6 (A-PCP)	F-BpsB-AT.FOR CTTTATTTTCAGGGCCAGTTGGGCAGGTTGG	Synthetic gene encoding BpsB module 6 (A-PCP)	-
	F-BpsB-AT.REV CCAAGCACTCTCGAGTTCACCCGCCAG		
	V-MBP.REV GCCCTGAAAATAAAGATTCTCAGAACCACT	-	pET-MBP-1d series
	V-MBP.FOR CTCGAGAGTGCTTGGAGTCATCC		
MbtH_BalH (non-tagged)	Fragm_balH.FOR GGAGATATACCATGGATGTGCAATCCGTTTCGACAACG	Synthetic gene encoding protein from balhimycin biosynthesis	-
	Fragm_balH.REV TTTACCAGACTCGAGTTAGGCACTTGCTTCG		
	V_pCDF.REV CCATGGTATATCTCCTTATTAAGTTAAACAAAATTATTTCTACAGG	-	pCDF (Novagen)
	V_pCDF.FOR CTCGAGTCTGGTAAAGAAACCGCT		

SI Table S5. Synthetic gene sequences of KisK module 5 (A-PCP-E-C), module 6 (A-PCP) and KisM protein, as well as primer sequences and template DNA used for cloning of wild type KisK module 6, the A domain KisK module 6 double mutant and MbtH-like protein

Synthetic gene names and sequences (restriction sites are underlined)
<p>KisK module 5 (A-PCP-E-C architecture)*</p> <p><u>CCATGGg</u>AGCAGCAGCAACCGCGACCCTGCCTCCGGTACCGGTCTTGTTCACCGTCAAGCCGAACGTCACCCGGGTG CGGTGGCCGTGACAGAAGATGGCCGCGATCTGAGTTACGCCGAGCTTGATGAAAGCGCAGGCCCGCTGGCTGCGTAT CTGGCCGGCCGCGGAGTGCGCCGTGGCGATCGCGTAGCGGTTGCGCTGGGTGCTCAGCAGATCTCGTGGTTGCATG GCTGGGCGTATGGCGTGCGGGTGCAGTGTTCTCGATCGACCCGGAATATCCGGCCGCTCGCGTGGCTTTTATGAT CCAGGACTCACGCCAGCGGCAGTGTTGTGTAGCGGACAGACGCGCAACTTAGTACGTGATCGTGACCCATATCGTAGT AGATGACCCAGCCATCCGTGCGGCAATTGCACAGGAGATCCGCTTAGTGTACCGTGTGGTGGTATGATCTTGCCTA TGTTATGTACACAAGCGGCTCGACCGGTACACAAAAGGCGTGGGTGTTCCCTCATGGCGCCGTTGCGGCACTGGTCG GCGAGCCGGGTTGGCATGTCGGTCCGGGTGATACGGTCTGGCCATGCCAGTCATAGCTTTGATATTAGCTTATATG AAATTTGGGTGCCACTCGCTGCAGGTGCGCGGTGCTGATTGCAGTCCCGGTGTTGTTGATGCTGGTGTGTCGCG ATGCAGTGCAGGTTGCGACCGCCGCTCATCTGACTGCCGGTCTTCCGGGTTTTAGCGGATGCCGCCCTGATT GCTTTCAGGCTGCGCAAGTTCTGACAGGTGGAGATGTGGTCCCGCTTGACGCTGTGCAACGTGTGCGTCTGGCGT GTCCGGCGGTGCGCGTACGTACATGTACGGCCGACCGAAACCACACTCTGCGCGACTTGGCAACTGATTGAGCCG GATGCGGAAATTGGTTCGGTGTACCGATTGGTTCGCGCCGCTGGTGGTTCGCGGGCCGTTAGTCTGGACCCGTTCTG AACCCGGTCCGCCGGGAATGATCGGTGAATTGACTTGGGTGGCGCCGGCGTTGCGCGTGGTTATCTTGGCCGCCA GGTCTGACGGGTGAGCGTTTCGTTGCCGACCGGGAGGTGAGCGTCTGTATCGCACTGGGGACCTGGCGAAATGGAC TCGGGATGGCGAATTGATCTTTCGCGGGCGTCCGACGCGCAGGTCAAAATTCGCGGCCACCGCGTCAACCCGGTG AAGTGGAGGCTGTCTTAGCAGCACATCCGGCAGTTCGACCCGAGTGGTGTGCGGGATGATGGTGCCTGATTGCCT ATGTGCTGCCCCGCGACAGGAAGATTCCGCCCTGACCACGGCGCTGCTGGATCACGCTCGCAAAGAATTGCCTGAAT ATATGGTCCCTGCTGCCGTAGTGTTCTCGATACGTTCCGCTTACCGTTAATGGGAAAGTCGACCGCGCAGCATTACC GGCACCGCTTTTCCGCTCAGGCGACCGGGGATCTGCCGGTTCGCGCATACGGTAACGAAACGGAACGCGTCTTAT GCGAACTGTTCCGCCGAGGTTCTGGGTCTGCCGGCAGCGGTGCGGGCTGAGGACAGCTTCTTACGCTGGGAGGTGAC TCAATCACTAGCATGCAGCTGACGGCTCGTGACCGCCGCGCCGGCTGCTTCTGCGTGTGAGGACGTTTGGAGCAC GCGACACCTGCAGGCAATTGCTGCAGTGAGCCGTGCGCGGATGAAGGCTCGGGTGGAGGCGAAGATGGCGCAGGTG AAATCGCTTGGACTCCTGTGATGCTGGACCTGATGGAAGTGGACAAAGAAAGTGTGCTGCGTGGGGAAGTGGCGCAG TGGGCGGTGGTTCGTGTGCCGGCGGGATTAACCCAGGACGTGTTGACAGATGGCTGGCAAGCGGTTCTGGATCGCCA TGACATGCTTCGGTTCGCGGTGGAGCCTGATGGGCGCTTAGTGGCGGCCCGGTTGGGGTGTGCGCCGCTCCGCGC AGATCGAACGCATTGAGGTAGATACCGCGGTTACAGGTGATGACCTGGATGCGTTAGCTCGTCAAGCTCACGAA GCAACTGGCCGTCTGGACCCCGCCGAGGCGTAATGGCGCGCTGGTATGGGTGCTGGGCGTGTGCGGGCTCGGAAA CGCGCCTGGCGGTTGCGGTGCATCATCTGGCGGTGATGGCGTCTCTTGGCAGATTCTGTTACCGGACTTGCCTGCGG CGTGTGAATCGCGCAAGCCGGCCGCGCCCCAGATTTGGCGCCGTTCCGACCTCCTTTCGCGCTGGGCACGCTTGT TGACCGAGCAAGCATCCGAACGCGCCGGTGAATTAGAAGCATGGCGCAGTATTTTGGCGACACGGACAGCCCGCTT GGGCGTGCCTCCGAACTGGCCGCGATACCGCGGCGACCGCGCGGCCATTATGACGTTAGCGGGCCCG AAGCTGTCAGCCTGGTGGACACGGCGACTGCCGCTTCACTGCGCGTGCATGAGGTTCTTAGCGGCGTTTTCGG GTGCGGTAGCGATGGTTCGCGGTGCGGCGGTAACGGTAGAAGTCAAGGCCACGGACCGCCCGTAGGGGAAGC CGATCTGACGCGCACTGTAGGTTGGTTCACGAGTGTTCATCCGGTGCCTTGGATGCTACAGGGCTGGATCACGGGCA AGCCCGCGCTGGCGGAGCAGCCCGGTCAACTCCTTAAGACCGTTAAGGAACAGGTGCGTGCTTTCCGGGAGACG GTTTGGGGTTCGGGCTGCTGCGTCATCTGAACCCCGAGACGGCTCCGTAAGTGGCAGGTTACCGCGCCACGTGTGG CGTTTAATTACATGGGGCGGTCCGCGGCGGCGAAAGCCGGTATGTGGCAGCCGGTAGACGCGATTGGCGGCAATGC CCATCCGGACATGCCACTCCGCCATGCTCTGGAAGTAGGGGCGAGTTCAGGATGGAGCCGACGGCCAGCTATGC ATCTGACGCTGACCTGGGCGAGGCGATCTGCTCGAAGAGGCTGAAGCCTTGGCATTGGGCCAGGCATGGCTTCTCTCC TGTCCGGTATCGCTGCGCATGCCGCGGCCCGGGCGCCGGTGGTACACCCCGAGTGATTTCCCGATGGTTGAGTTAG ACCAGGCGGAGGTGACGAAGTGGATGAAGTGTCCGGGCGCCGACTGGCGGATGTCTGGCCGCTCTACCACTGCAG GAAGGTTTACTGTTTACGCGAGCTATGACGCTTCTGCCGGGACGCTACGAGTGCAGCGCGTCTCGATCTGACA GGGCTCTGATGCCGCGCGCTGCGTGCGGCTTGGGAGAGCGTTTTAGCCCGTCATGACGCGCTGCGCGCGGGCTT CCACCAGTTGGGCTCTGGCCGTGCGGTGCAGGTAGTTGAGATCGTGTGCAACTGCCGTGGCGTACCCTTGGTGGC GCTCTGCGGGAAGCCGAACGTGCCCGCGGGAAGAAGTACCGAAGCTTTGATCTGAGCGAGGCTCCGTTGCTT CGTGTGCTCCTCATCCGTCTGGCGGAGCGCCGCATCGTCTGGTGGTACGCTCTACCATATTATTGGGATGGCTGG</p>

Synthetic gene names and sequences (restriction sites are underlined)

TCCCTTCTGTCTTATTTGGTGAAGGTGAAAGCTGCATACGATGATCCAGGCACTGCCGGCCCGGGCGGAGCTATCGT
 ACGTATCTGGAATGGCTGGAACGCCAAGACGCGGAGGCTGCACGTGCGGCCTGGCGCATGAACTGGGTGATCTCG
 ACGAGCCAACCCGTGTTGCCCGGAGAAACCGCCACTGTGGCGGCCCGTCCGGAACGGCTGCGTTACGAACTGACG
 GGCAATTGTCTGGGGCGATGAGTCGCTGGCACAAGCCCAAGGAGTGACGATGAACACAGTACTGCAAGCCGCTG
 GGCCTGTTCTCGCACGCTAACGCGCCGTACCGATGTCGTGTTTGGCATGACTACCGCCGGCCGTCCAGCGGAACT
 CCCGGCGGTGAGCGCATGGTGGGCTGTTCACTATGGTGCCCGTCCGTGTGCGGCTGCGCGGGACCACCACCG
 TGGCTCGTCTGTGACCGAGGTACGCGAACGCCAGATTGCGTTGATGCCTCACCAGCACCTTGGCCTCGCGGAAATTC
 AACGGACCGCCGGCCAGGGGCTTCGTTTACTCAACCGTGGTTTACGAGAATTATCCACGCCACCCTGGATTCTCC
 GGGTCCCAGCGCTTACCATGCGGCCTGCGGGCGTTCAGAGGACGCGGGCCATTATCCACTGACCTTAGTGGCTGC
 ATTCGATGATGGCCGTGTGGTTGGCGATCTGATCTATCGCCCGATGCTTTTGGTTCGCGAACGTGCCGAGTTAGTGT
 GGCGGCCCTGGTCCGCTTCTGGAACGCATGACCGCCGATCCTGCAGGCACCGTGAGCCGCATTGGCCTGGATGGAC
 CAGGCGTGAACCGCCCTGGACCTCAGGCCTCGAG

KisK module 6 (A-PCP architecture)

CCATGGgACCACGTGCAGCTGGGGAAGCGCCGTTAGGGGAGTTAATTCGCCGCATTGCCACGGAACGTCCCGAGGCG
 GTGGCAGTAGTGGATGGCGATGGGGAACCTACGTACGCCGAGCTCCTGACACGTGCGACGGGATTAGCACGCCACTT
 GGTGCGTCTGGTGTGCCCCGGAACGTGCGTTCGAGTCTGTTGAACGGGGCGCCGCATGGGTACCCGCCGTAC
 TGGGCGTTGCCCTGGCGGGCGTGTGAGTGTGCTGGAACCAAGCTATCCGCCTGAACGCTTAGGCTGGATGCTG
 AGTGATAGTGCGCCACAGTCGTGGTTGCTCTGCAGCGACGCGTTTTCTGTCCCGGCTGGGGTGGATACCGTTGTA
 GTCGATGCGGGCGTAGCCGACGGGCTCACCAGCGAGGCCCGCTGCCGAAGTGACCCCTGAACACGCGGCCTATGT
 GGTGTACACGTGCGGCTTACCGGGTTACCTAAGGGCGTTGTGGTCAGCCATGCCGGGTTGGCCAATCTTGTACTGC
 TCAGATTGATCGTTTGGGGTCACTCCGAGCTCGCGTGTACTCCTGATGGCTGCGCTCGGATTCGATGCGGTGATGC
 GGAAGTGTGATGGCCCTGCTGAGCGGTGGAACCGTGGTTGCACTGCCGGGGTATGAACTGCCACCGCGTACCGGTC
 TGGCTGAAACGCTGCTGCGCTGGGGTATCACCCAGTTACCGTCCCGCCGAGTGTGCTCGCCACCGTTGCAGAAGATC
 TGCCGGAACCGTTCGAGACAATCGTTGTTGCCGGAGAAGCGTGTGGTCCAGACTGGTTCGAGCGCTTCTCACCTCGCC
 ATCGGATGGTCAACGCGTATGGACCGTACAGAGGCCACTGTCTGCTCGACCATGTCCGGACCATGGCGCCCGGCGG
 GAAGTACCGATTGGAACCTCCGATTGACAGGCGGGCGCTGCGAAGTCTTGATCAGTTTCTGCGTCCGCTTCTCCCGGT
 GTTACCGGGGAGCTGTATGTAGCGGGCGCCGGCTGGCGCGCGGTTACCTGAATCGCCCGGCTGACGAGTGCACG
 CTTTGTGGCGGACCCGATGGTCCCGTCTTATCGCACTGGTGTGCTGGCCTACTGGACACTGGTGGTGAACCTGGT
 GTTCGTAGCCCGCGCGGACGAAACAGGTCAAAGTGCAGCGTTTTCTGCGTGGAAACCGGGCGAGATCGAAGCCGCGATT
 ACGGCACATCCGCATGCGGCGCAGGCCGCGGTGGTGTGTGGCGTGGCCGCTGGTTGGCTACGTTGTACCAGCTCC
 GGGTGGCATTACCGCAGATGAAGTTCGTGAACACCTTGTGCGCGTTTACCGGGCCACATGGTGGCGCCGCTTATTAT
 GGTACTGGAAGCACTGCCGCTACCCCAAACGGCAAAGTGGACCGGAAAGCGCTTCCGGAGCCGGAATTGCGAGCAG
 GCGGCGGCGATGCTGAACGGGCGCAGGTGCGCCAGCGACCGAAACGGAACGTGCGTTGTGTGAGCTGTTTGCAGA
 GGCGTTGGGCGTTGATCGCGTGGGTGCTGACGATAACTTCTTTGACCTGGGCGGCGACTCCATCATCTCCATGCGGTT
 AGCTGCCCGTGCACGCCGAGCGGCCTGACACTGTCTCCCGCCAAGTGTTCGAGGAGAAAACCCACGTCGCTTAGC
 GGCCTTAATTGACGCTTATCGACGCTGCCCTCCTCCGCCGCTCCGGGCCAGGCGCGGCCGCGTTCGCTCAGGT
 GCGCCGCTTGTGGAATTGACTGGCGACCAACTGGCGGAATTGGAAGCGGCGCTGGGTGCCGGTCTGGTCATGATGA
 TGCGCCGGGTGCAGGTCCGGGTGATGACGATGCGCCAGATGAAGGTGCACAGCGCCTCGAG

KisM (MbtH-like protein)

ATGACCAATCCGTTTACGACGTTGATGGGACGTTCTTCTGTTGGTGAACGACGAAGGCCAACACAGCTTATGGCCG
 GTATTTGCGGAGATTCCGCAAGGTTGACTGCCGTGTATGGCGAAGCTGGACGTGAGGAATGCCTGCAGTACGTGGA
 AGAGCATTGGACCGATATGCGCCCTAAATCGCTGGTTGCCAGATGGATGGTTCGAGTCCAGATGCACGTGCGTAA

Final construct	Primers	Insert template DNA	Vector template DNA
Module 6 (WT) A-PCP	M6AT-Kis-fwd TATTTTCAGGGGCCACAGTAAGCAGAATAGGACTAGACGGACCA GGAGTAGAACAGCATGGACAAGCGGAGgACCACGTGCAGCTGG G	Synthetic gene encoding KisK module 6 (A-PCP architecture)	-
	M6AT-Kis-rev CCAAGCACTCTCGAGGCGCTGTGCACC		
	Vector-M6AT-fwd CTCGAGAGTGCTTGGAGTCATCC	-	pET-GB1-1d series

Synthetic gene names and sequences (restriction sites are underlined)			
	Vector-M6AT-rev GGCGCCCTGAAAATAAAGATTCTCAG		
Module 6 (A-PCP, A domain double mutant)	F1-M6A(x2aMu)T-Kis-fwd AGAAGGAGATATACCATGAAACATCACCAT	pET-GB1-1d, encoding KisK module 6 (A-PCP architecture)	-
	F1-M6A(x2aMu)T-Kis-rev GATTGTCTCGACCGTTTCCGGCAGATC		
	F2-M6A(x2aMu)T-Kis-fwd ACGGTCGAGACAATCATAGTAGCAGGAGAAGCATGCGGACCAGA CCTAGTAGAAAGATTTCAGCCCAAGACACAGAATGGTAAACGGATA TGGACCGTCAGAGGCC		
	F2-M6A(x2aMu)T-Kis-rev GCAGCCGGATCAAGCTTACTTCTCGAA		
	Vector- M6A(x2aMu)T-fwd GCTTGATCCGGCTGCTAACAAAG	-	pET-GB1- 1d series
	Vector- M6A(x2aMu)T-rev GGTATATCTCCTTCTTAAAGTTAAACAAAATTATTCTAGAGGG		
KisM (non- tagged)	MbtH-Kis-fwd GGAGATATACCATGGATGACCAATCCGTTTGACGACG	Synthetic gene encoding KisM protein from kistamicin biosynthesis	-
	MbtH-Kis-rev TTTACCAGACTCGAGTTACGCACGTGCATCTGGACTG		
	Vector-pCDF-fwd CTCGAGTCTGGTAAAGAAACCGCT		pCDF (Novagen)
Vector-pCDF-rev CCATGGTATATCTCCTTATTAAAGTTAAACAAAATTATTCTACAGG	-		

*KisK module 5 (A-PCP-E-C) synthetic gene was used for Tcp11 module 5 hybrid construction.

See **SI Table S6**.

SI TableS6. Primer sequences and template DNA used for cloning of Tcp11 module 5 hybrid

Final construct	Primers	Insert template DNA	Vector template DNA
Tcp11 module 5 hybrid (A-PCP-E-C*)	F1-Tcp11M5ATE-fwd AATCTTTATTTTCAGGGGAGCAGCGTA	pET-GB1-1d, encoding Tcp11 module 5 (A-PCP-E-C architecture)	-
	F1-Tcp11M5ATE-rev ACCATCGGGAAATCGCTTGGGGTATG		
	F2-KisKM6C-fwd CGATTTCCCGATGGTTGAGTTAGACCAG	Synthetic gene encoding KisK module 5 (A-PCP-E-C) (See Supplementary Table 4)	
F2-KisKM6C-rev GCAGCCGGATCAAGCTTACTTCTCGAA			
	V-M5Hybrid-fwd GCTTGATCCGGCTGCTAACAAAG V-M5Hybrid-rev CTGAAAATAAAGATTCTCAGAACCACTGC CA	-	pET-GB1-1d series

*C domain was replaced with analogous KisK C domain.

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