
Supplementary information

A natural depsipeptide antibiotic binds the E-site of the bacterial ribosome

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Supplementary Information

A Natural Depsipeptide Antibiotic binds the E-site of the Bacterial Ribosome

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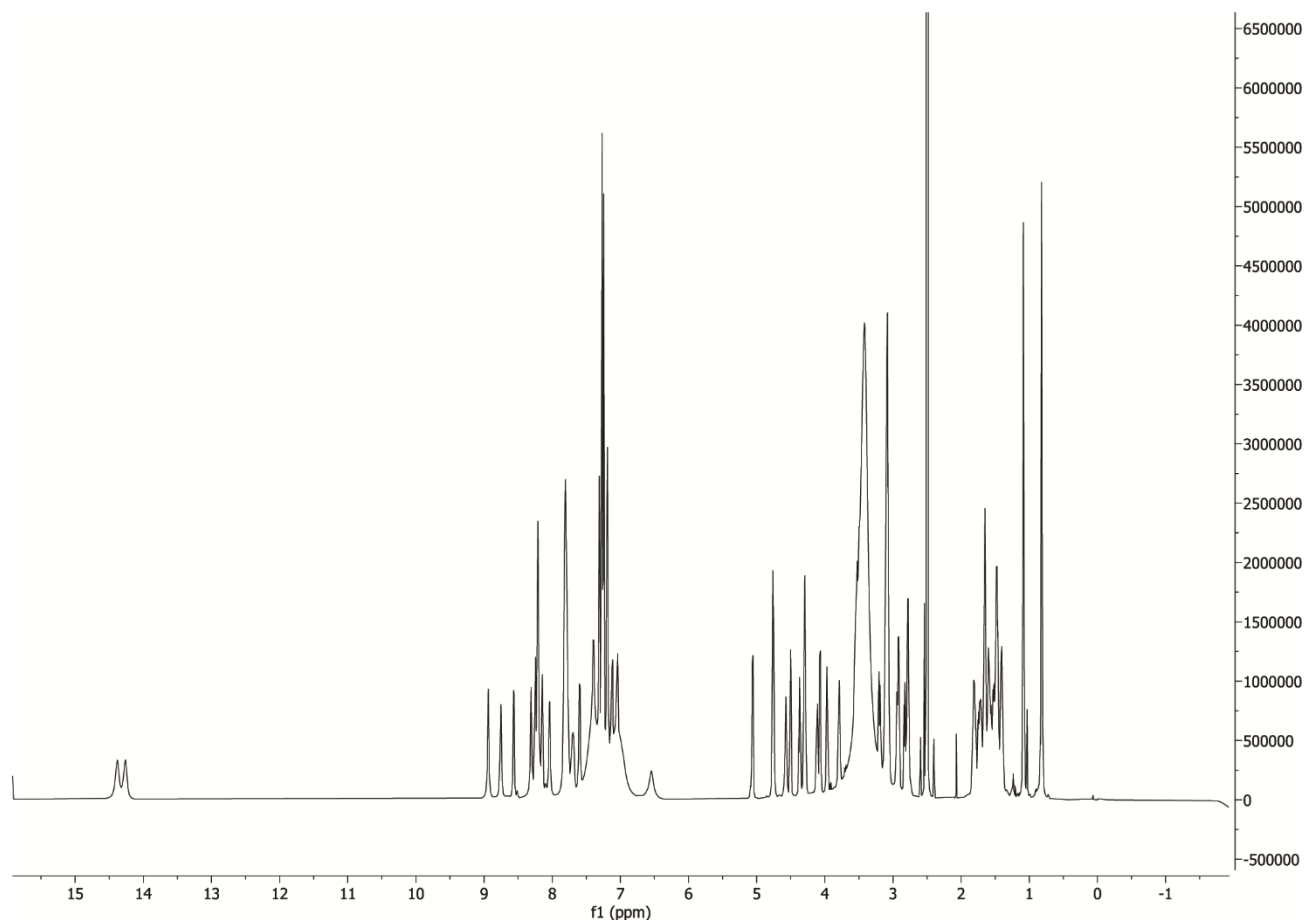
[#] correspondence to: wrightge@mcmaster.ca, shura@uic.edu, daniel.wilson@uni-hamburg.de

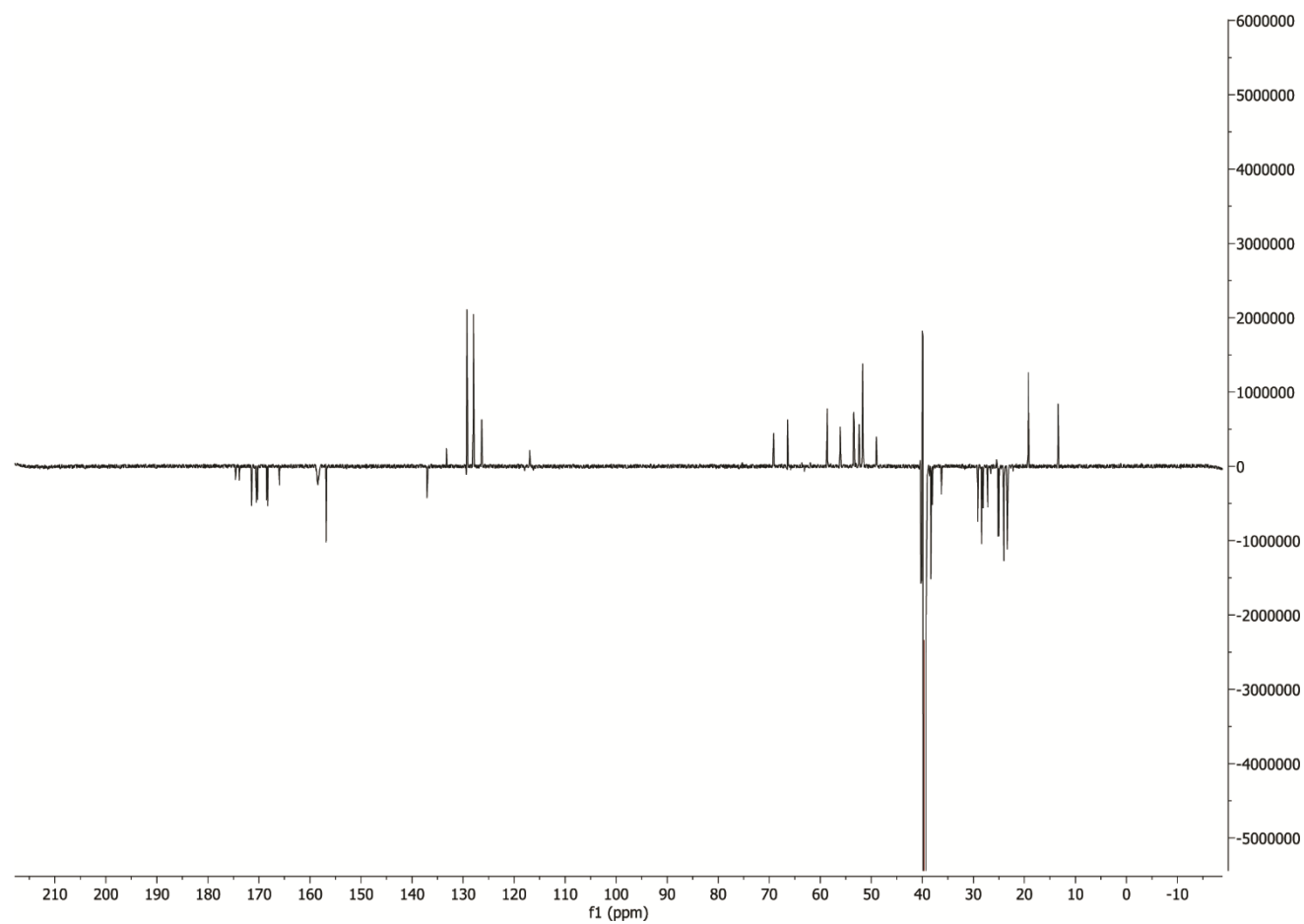
Keywords: Depsipeptide; NRPS; ribosome; antibiotic; E site; translocation; gram-negative bacteria; antimicrobial resistance.

Table of Contents

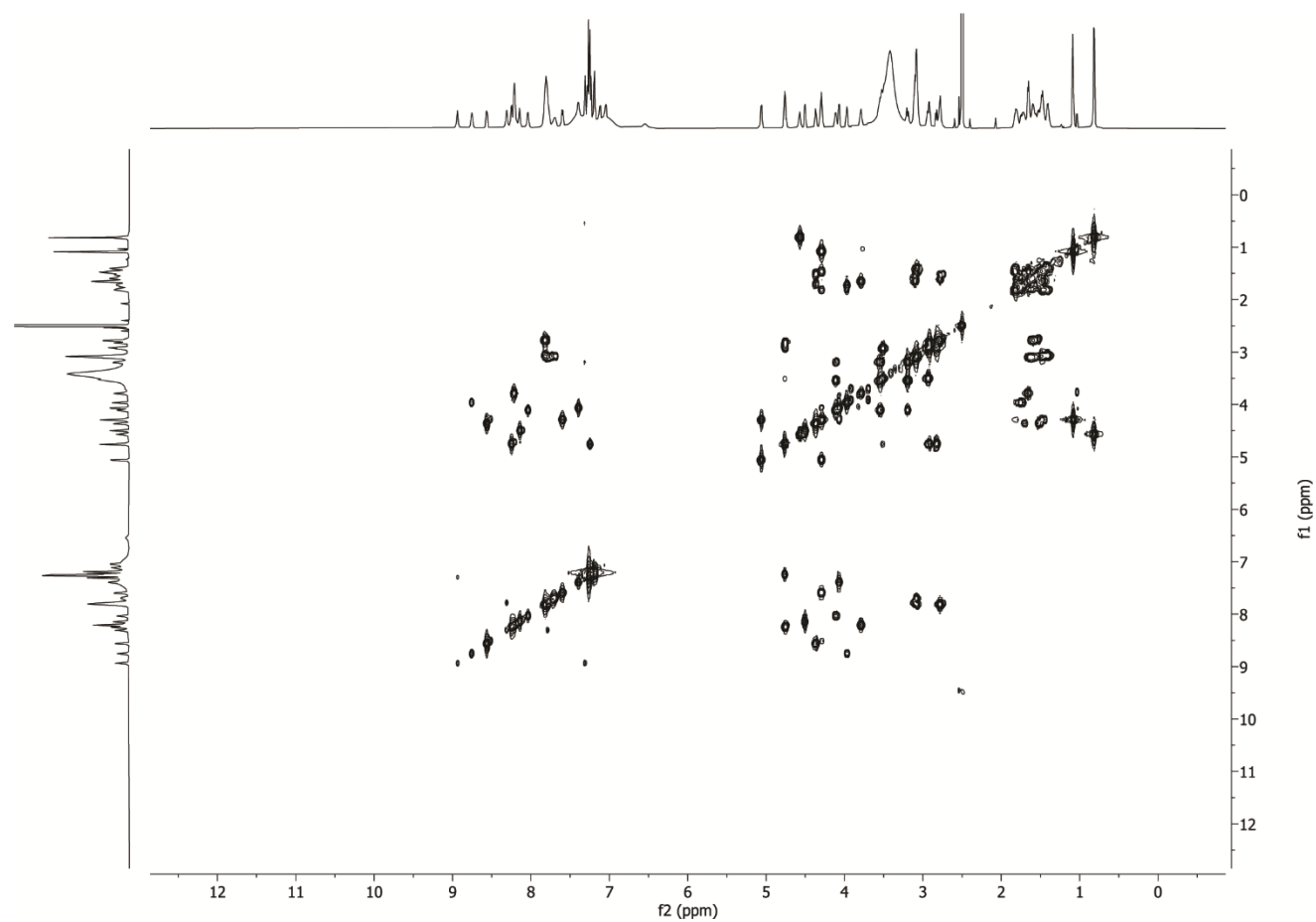
SUPPLEMENTARY DATA FIGURES	4
Supplementary Fig. 1 ^1H NMR spectrum of MKM-A.....	4
Supplementary Fig. 2 ^{13}C DEPTQ NMR spectrum of MKM-A.....	5
Supplementary Fig. 3 ^1H-^1H COSY NMR spectrum of MKM-A.....	6
Supplementary Fig. 4 ^1H-^{13}C HSQC NMR spectrum of MKM-A	7
Supplementary Fig. 5 ^1H-^{13}C HMBC NMR spectrum of MKM-A.....	8
Supplementary Fig. 6 TOCSY- HSQC NMR spectrum of MKM-A.....	9
Supplementary Fig. 7 NOESY-NMR spectrum of MKM-A.....	10
Supplementary Fig. 8 NMR assignment of MKM-A in deuterium oxide (D_2O).....	11
Supplementary Fig. 9 ^1H NMR spectrum of MKM-B.....	12
Supplementary Fig. 10 ^{13}C DEPTQ NMR spectrum of MKM-B.....	13
Supplementary Fig. 11 ^1H-^1H COSY NMR spectrum of MKM-B.....	14
Supplementary Fig. 12 ^1H-^{13}C HSQC NMR spectrum of MKM-B	15
Supplementary Fig. 13 ^1H-^{13}C HMBC NMR spectrum of MKM-B	16
Supplementary Fig. 14 TOCSY- HSQC NMR spectrum of MKM-B.....	17
Supplementary Fig. 15 NOESY-NMR spectrum of MKM-B	18
Supplementary Fig. 16 ^1H NMR spectrum of MKM-E	19
Supplementary Fig. 17 ^{13}C DEPTQ NMR spectrum of MKM-E.....	20
Supplementary Fig. 18 ^1H-^1H COSY NMR spectrum of MKM-E	21
Supplementary Fig. 19 ^1H-^{13}C HSQC NMR spectrum of MKM-E.....	22
Supplementary Fig. 20 ^1H-^{13}C HMBC NMR spectrum of MKM-E	23
Supplementary Fig. 21 TOCSY- HSQC NMR spectrum of MKM-E.....	24
Supplementary Fig. 22 NOESY-NMR spectrum of MKM-E	25
Supplementary Fig. 23 Extracted Ion Chromatogram (EIC) of acid-hydrolyzed MKM variants treated with Marfey's reagent.	26
Supplementary Fig. 24 Mass spectrometry analysis of MKM variants.	27
Supplementary Fig. 25 (a) Yield proportions of the MKM variants. (b) HPLC chromatogram displaying the peaks corresponding to the individual MKM variants...	28
Supplementary Fig. 26. In silico sorting scheme of the E. coli MKM complex.....	29

Supplementary Fig. 27. Angular distribution and local resolution of MKM cryo-EM maps.	31
Supplementary Fig. 28. Secondary binding site of MKM on the 50S subunit.	32
Supplementary Fig. 29. Interaction of MKM in the E-site of the 50S subunit.	33
Supplementary Fig. 30 tRNA identity and codon-anticodon interaction.	34
Supplementary Fig. 31 BLAST analysis of the MKM BGC.....	35
Supplementary Fig. 32 Stachelhaus code analysis.	36
Supplementary Fig. 33 Conserved binding mode of MKM congeners	37
Supplementary Fig. 34 Map of the pCGW vector with MKM BGC.	38
Supplementary Fig. 35 Plasma stability and pharmacokinetic characterization of MKM-A.	40
Supplementary Fig. 36 Role of peptide uptake transporters SbmA and YejABEF in MKM susceptibility in E. coli.	41
Supplementary Fig. 37 Raw blots for toeprinting experiments used in Fig. 4b,c,f.....	42
Supplementary Fig. 38 Raw blot for toeprinting experiment used in Fig. 5b.	43
Supplementary Fig. 39 Raw blots for toeprinting experiments used in Extended data Fig. 4 a, c.....	44
SUPPLEMENTARY TABLES	45
Supplementary Table 1 ¹H and ¹³C NMR assignment of MKM-A	45
Supplementary Table 2 ¹H and ¹³C NMR assignment of MKM-B	46
Supplementary Table 3 ¹H and ¹³C NMR assignment of MKM-E	47
Supplementary Table 4 Calculated and Observed Masses of MKM variants.	48
Supplementary Table 5 Proposed functions of proteins encoded by the MKM BGC (accession no: JBPBLP010000002.1) using antiSMASH v6.0.0³ and antiSMASH v8.0.0⁴	49
Supplementary Table 6 Mutations in MKM-resistant E. coli mutants from serial passaging	50
Supplementary Table 7 Bacterial strains and plasmids used in the study.	51
Supplementary Table 8 Oligonucleotide primers used in this study.....	53
Supplementary Table 9 mRNA templates used in the study.	54

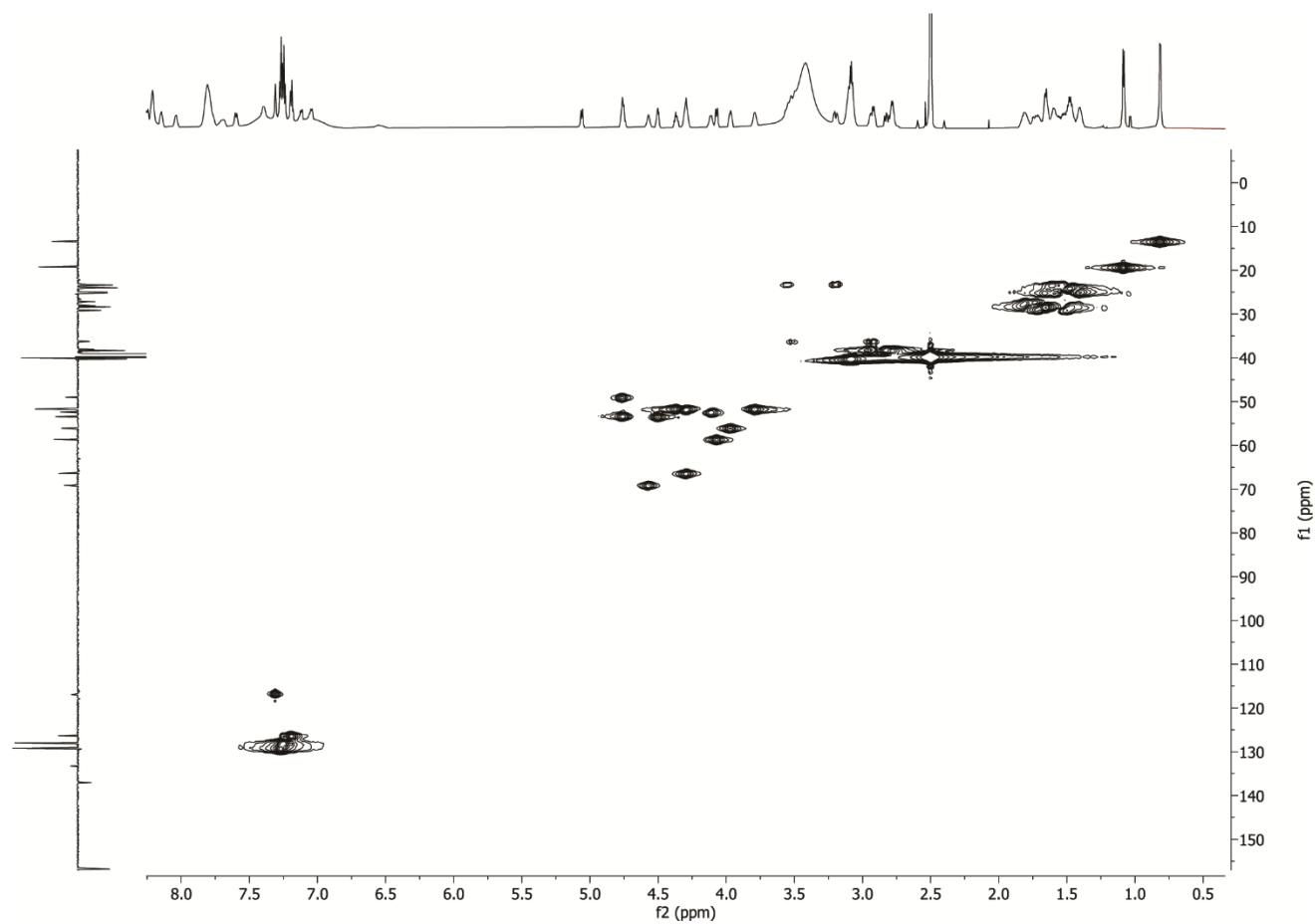
SUPPLEMENTARY DATA FIGURES**Supplementary Fig. 1 | ¹H NMR spectrum of MKM-A**



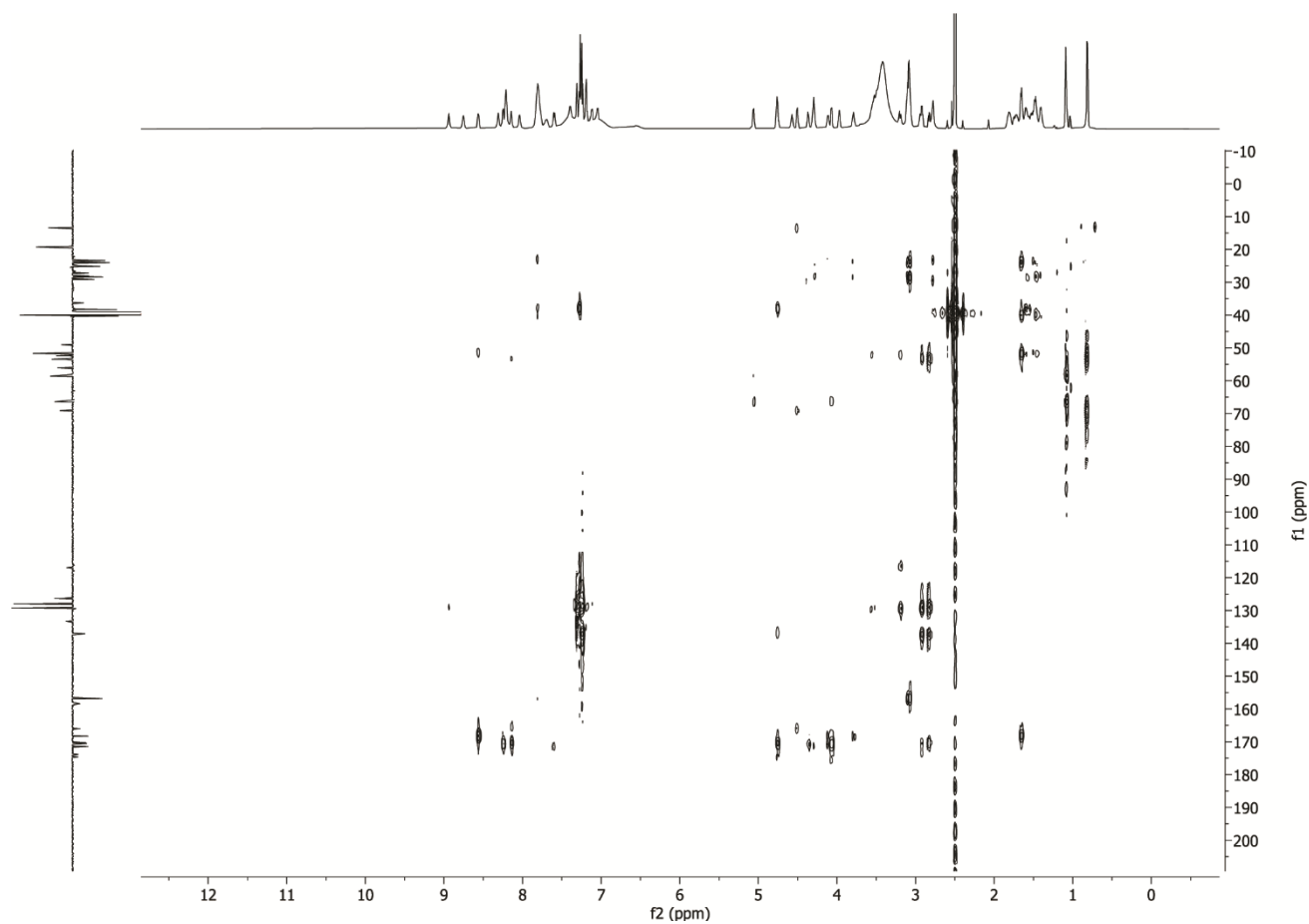
Supplementary Fig. 2 | ^{13}C DEPTQ NMR spectrum of MKM-A



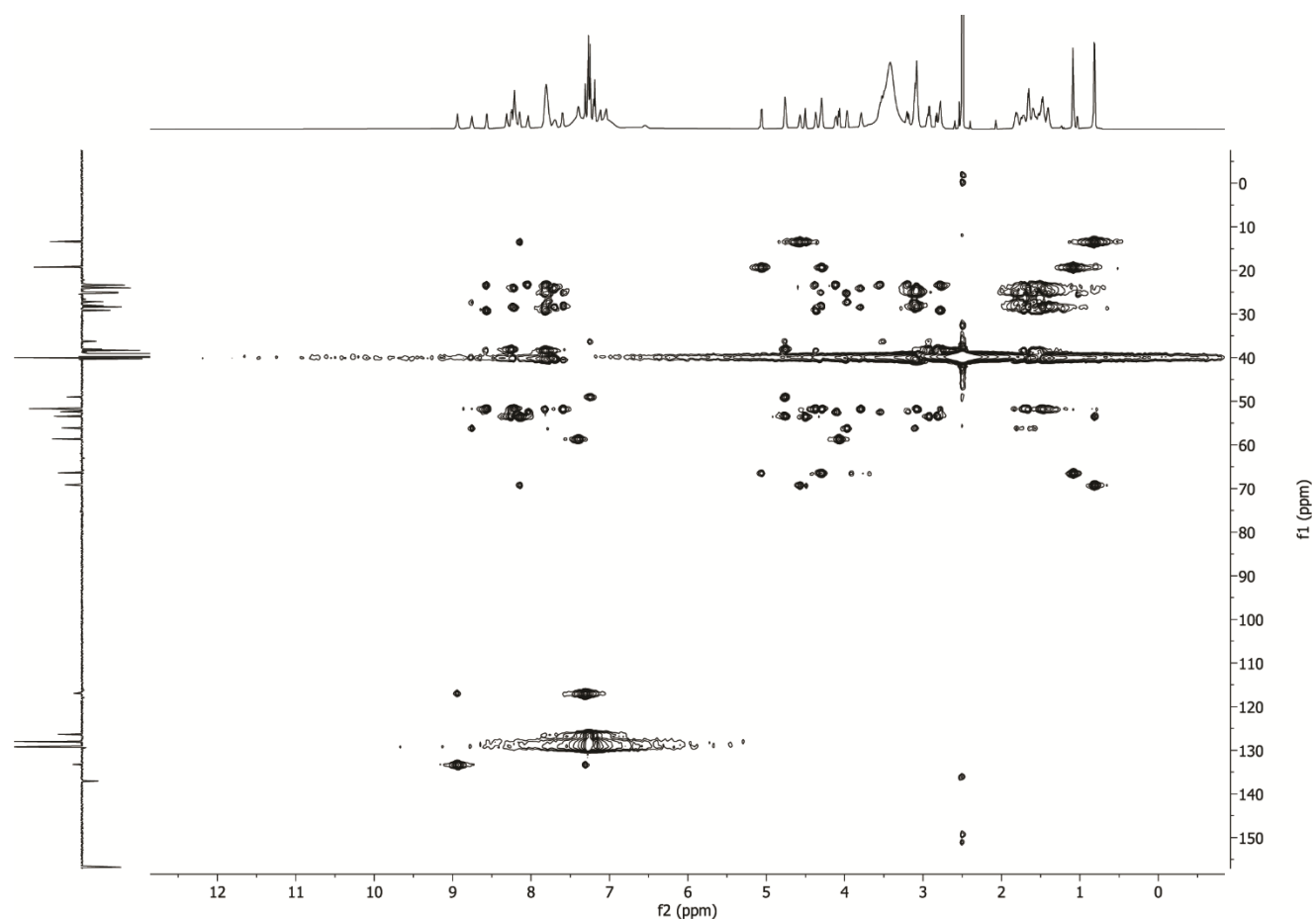
Supplementary Fig. 3 | ^1H - ^1H COSY NMR spectrum of MKM-A



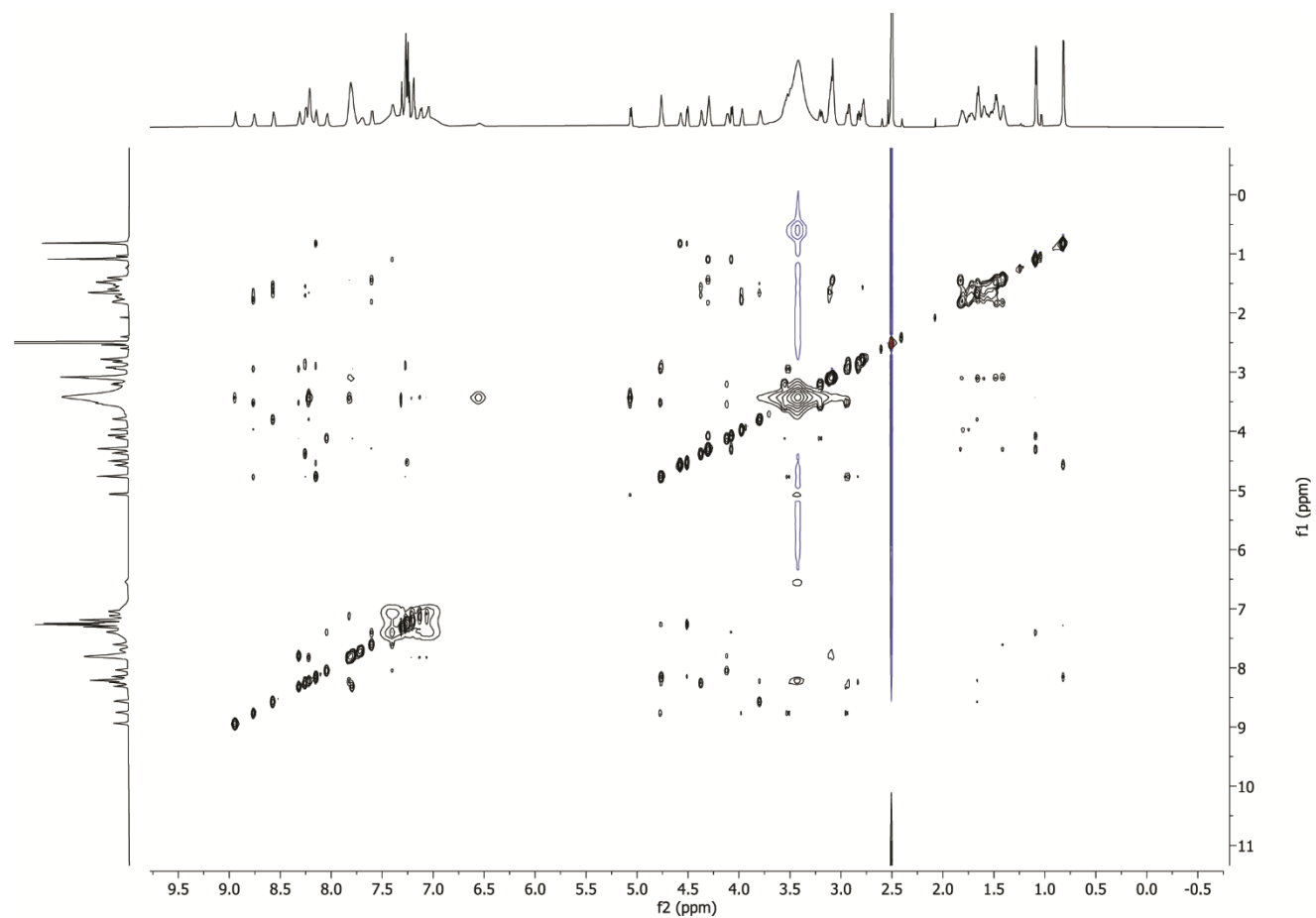
Supplementary Fig. 4 | ^1H - ^{13}C HSQC NMR spectrum of MKM-A



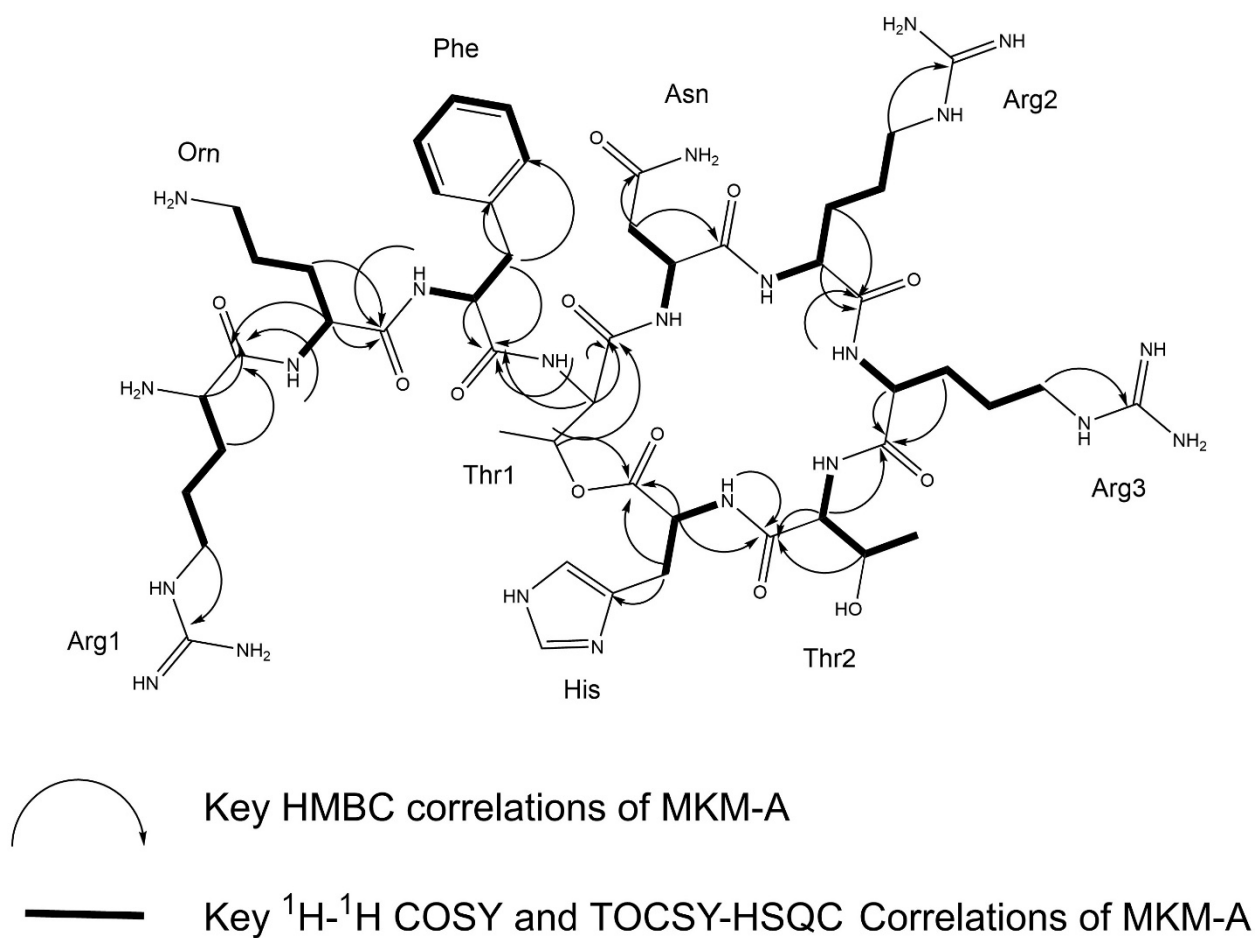
Supplementary Fig. 5 | ^1H - ^{13}C HMBC NMR spectrum of MKM-A



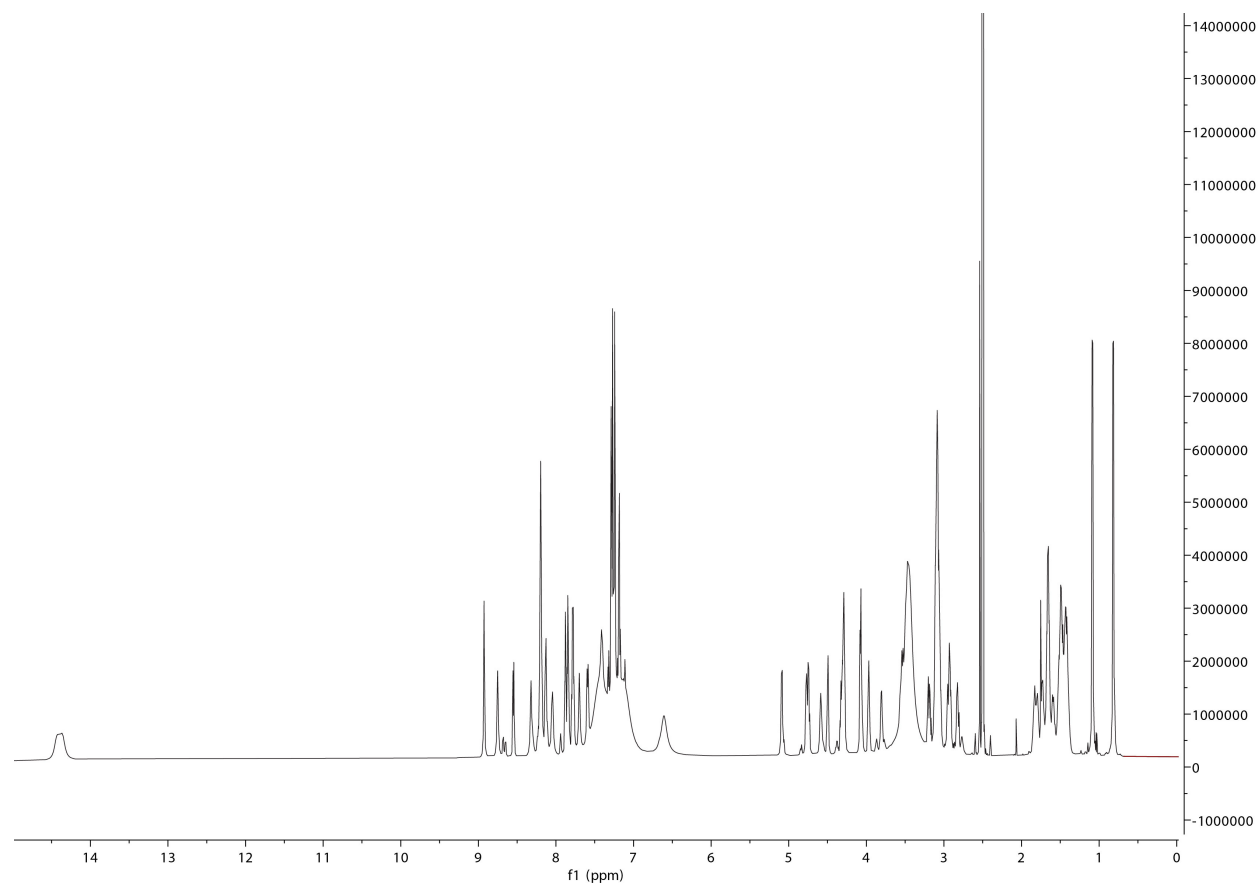
Supplementary Fig. 6 | TOCSY- HSQC NMR spectrum of MKM-A



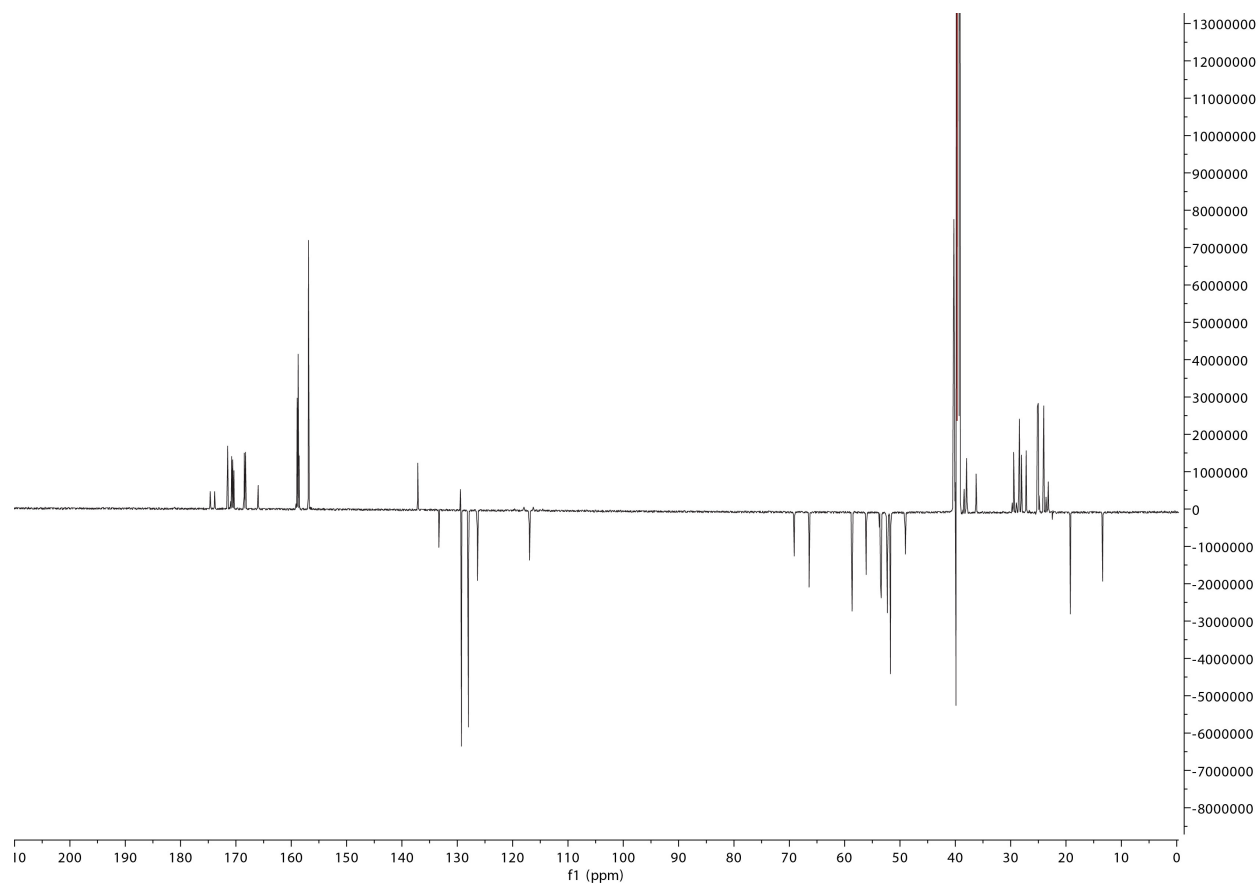
Supplementary Fig. 7 | NOESY-NMR spectrum of MKM-A



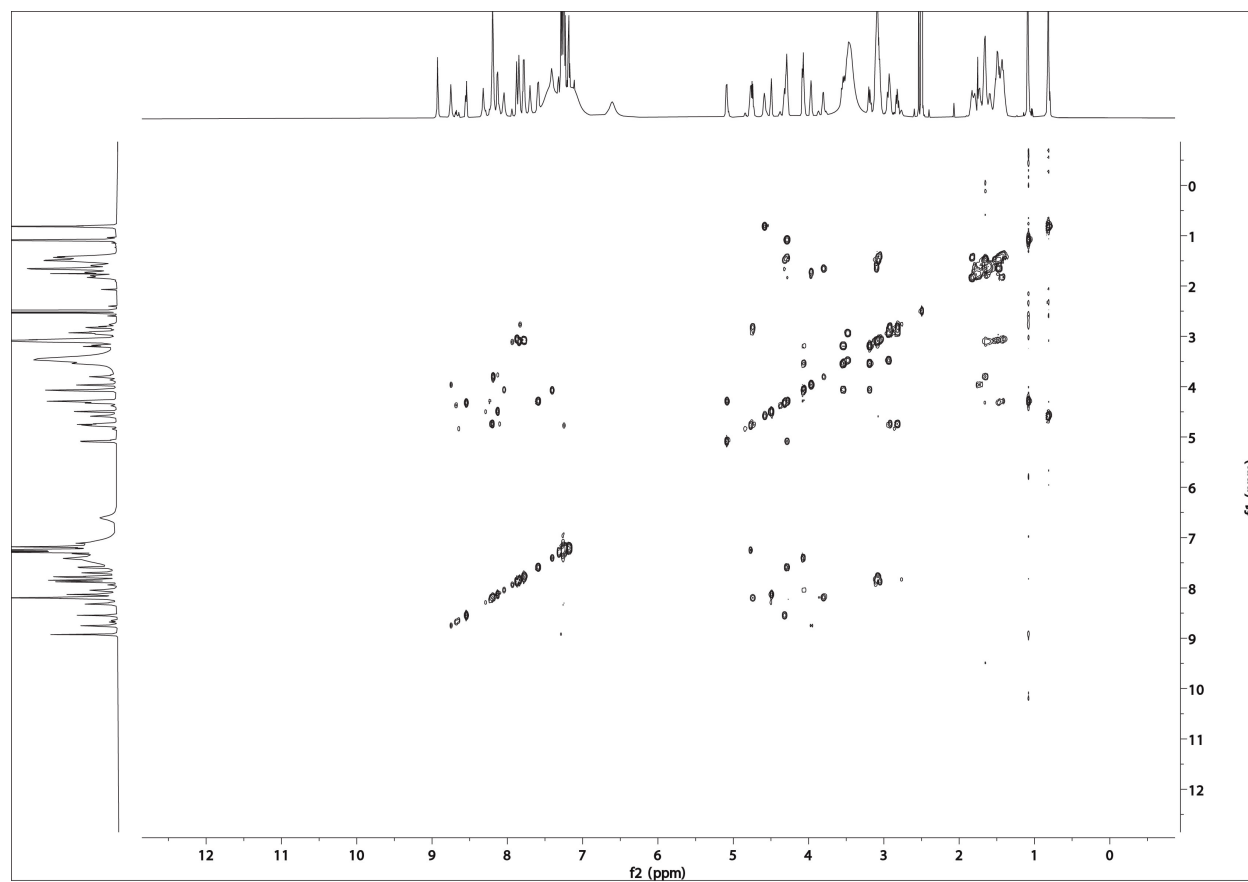
Supplementary Fig. 8 | NMR assignment of MKM-A in deuterium oxide (D₂O). ^1H and ^{13}C NMR chemical shifts of various amino acids in MKM. The key correlations of MKM-A relevant to NMR spectra are shown.



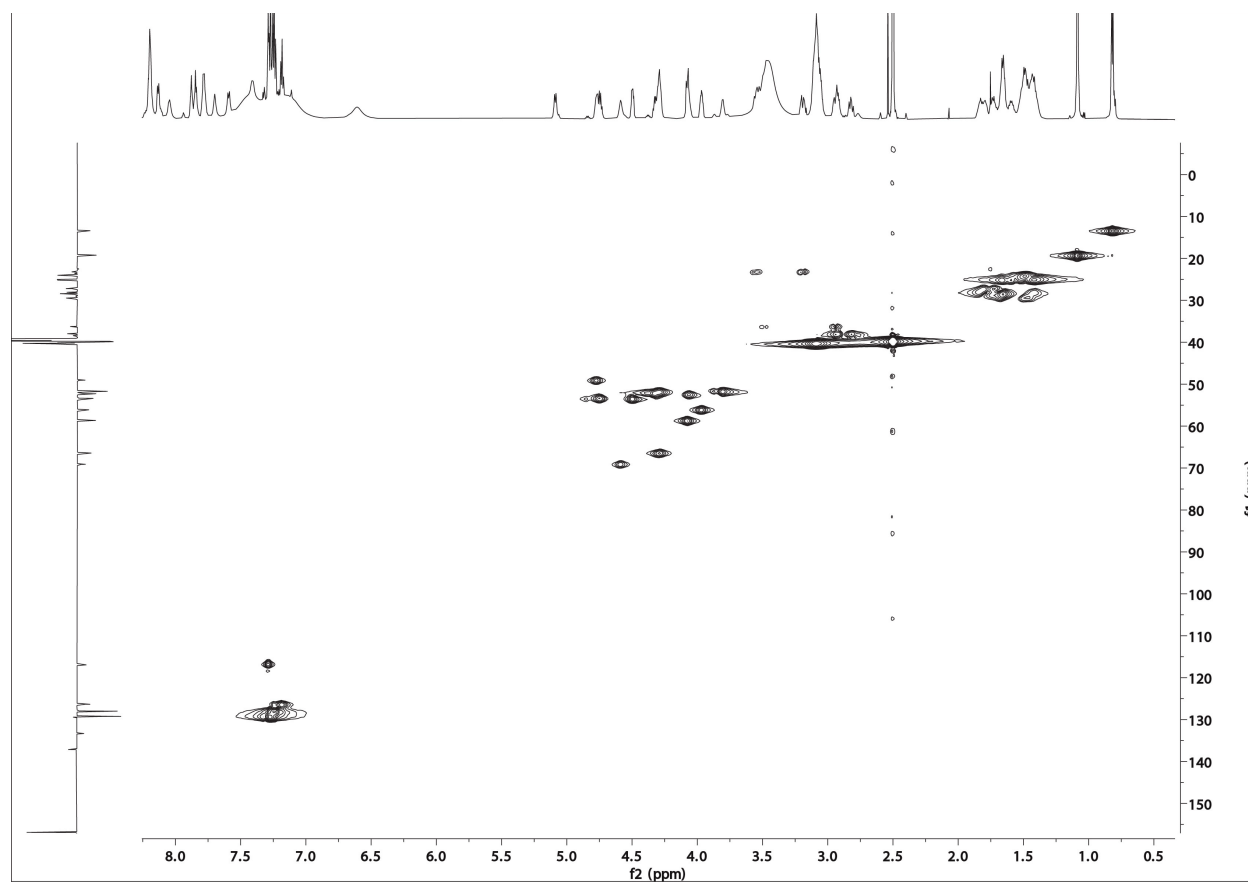
Supplementary Fig. 9 | ^1H NMR spectrum of MKM-B



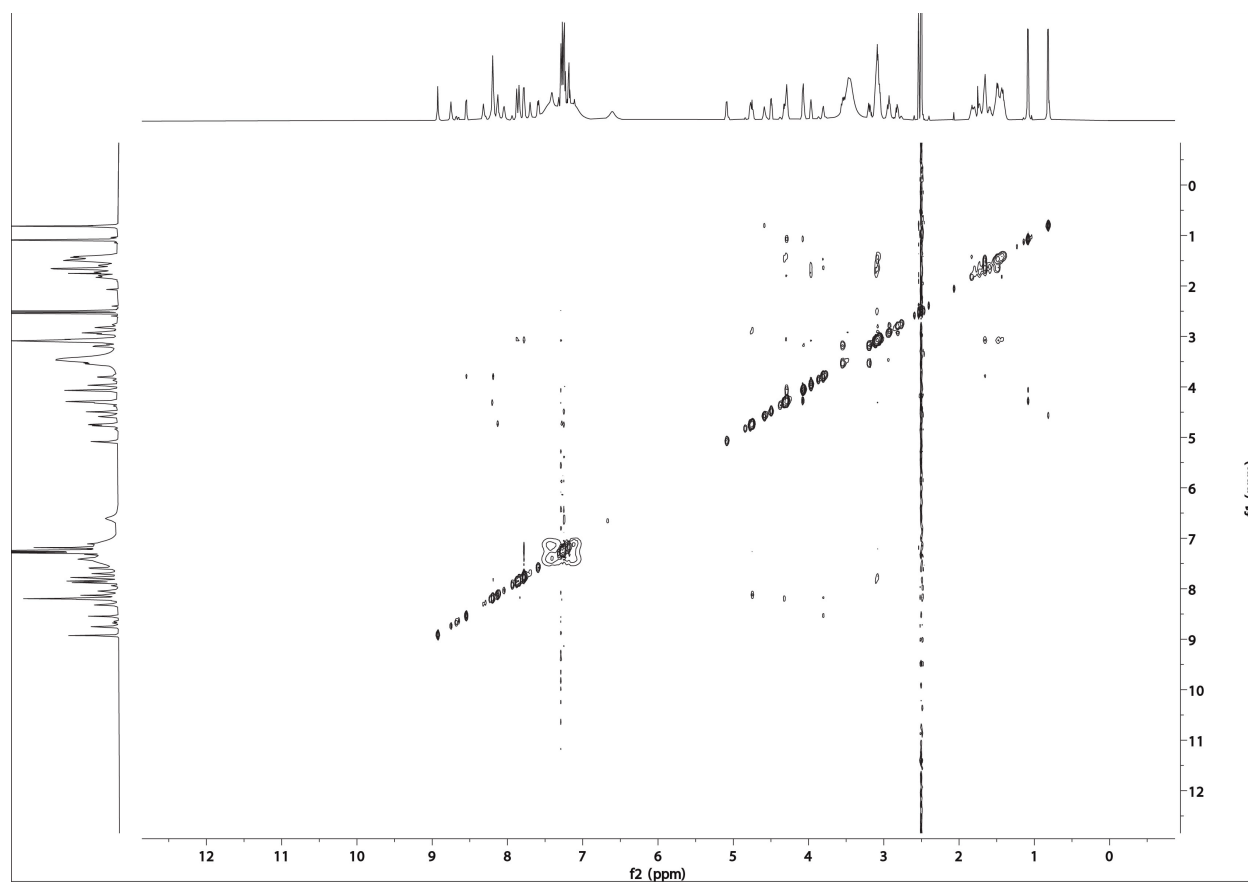
Supplementary Fig. 10 | ^{13}C DEPTQ NMR spectrum of MKM-B



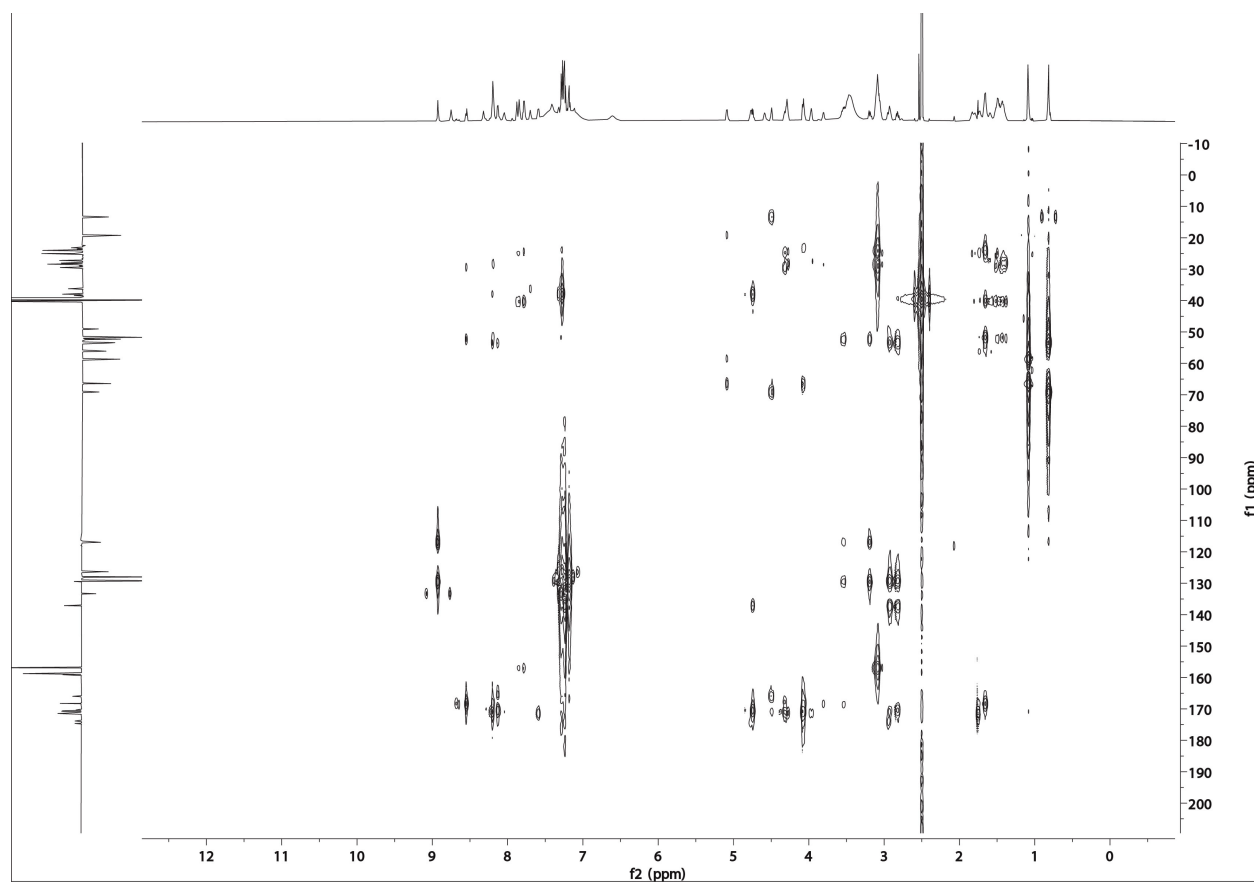
Supplementary Fig. 11 | ^1H - ^1H COSY NMR spectrum of MKM-B



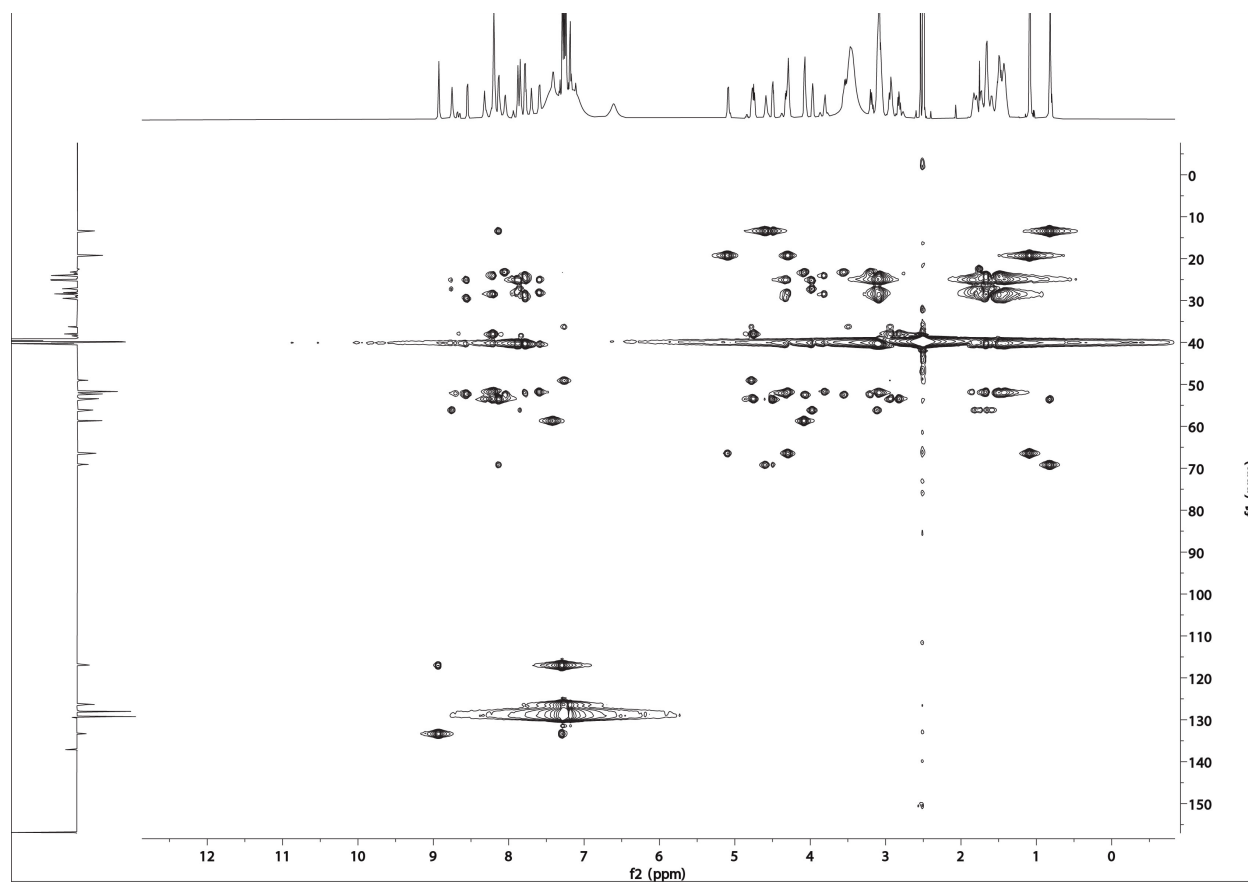
Supplementary Fig. 12 | ^1H - ^{13}C HSQC NMR spectrum of MKM-B



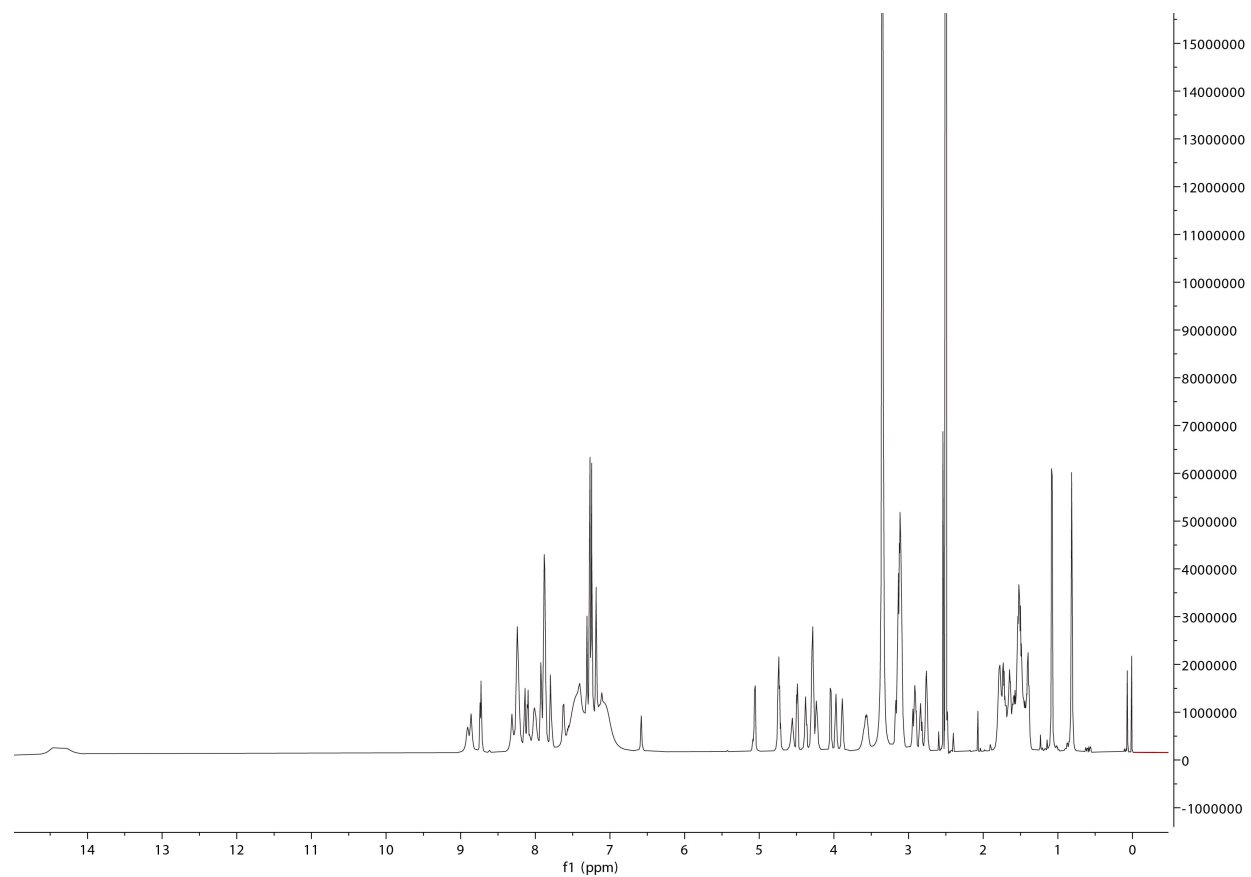
Supplementary Fig. 13 | ^1H - ^{13}C HMBC NMR spectrum of MKM-B



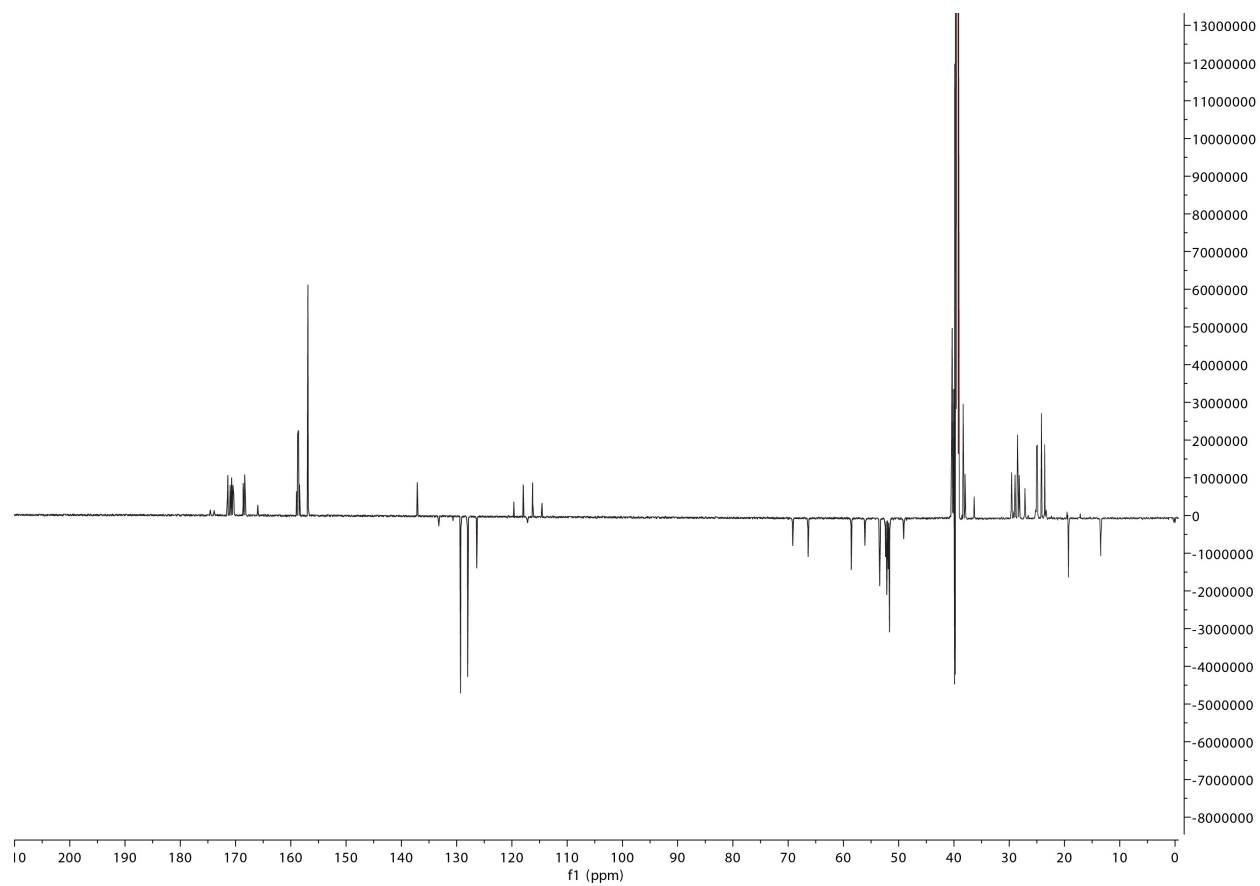
Supplementary Fig. 14 | TOCSY- HSQC NMR spectrum of MKM-B



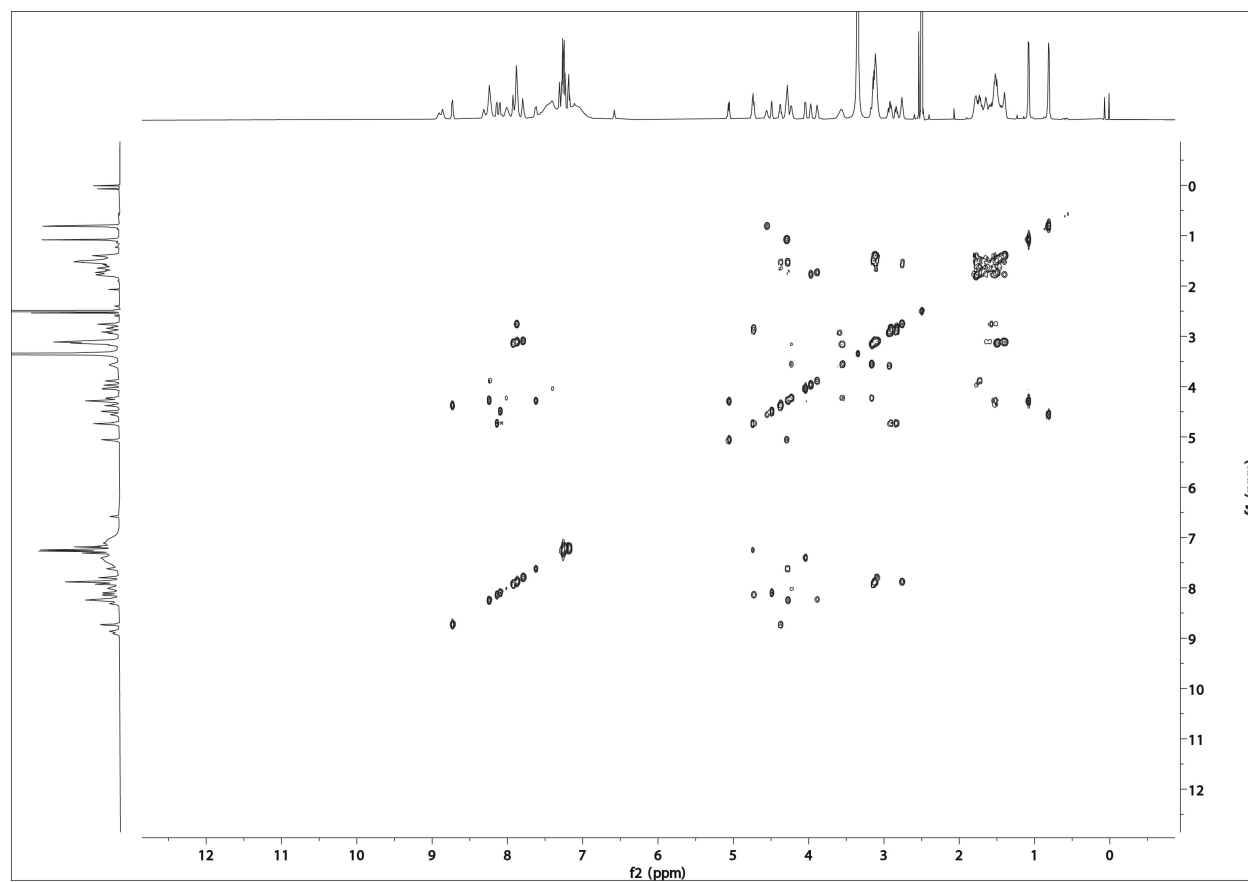
Supplementary Fig. 15 | NOESY-NMR spectrum of MKM-B



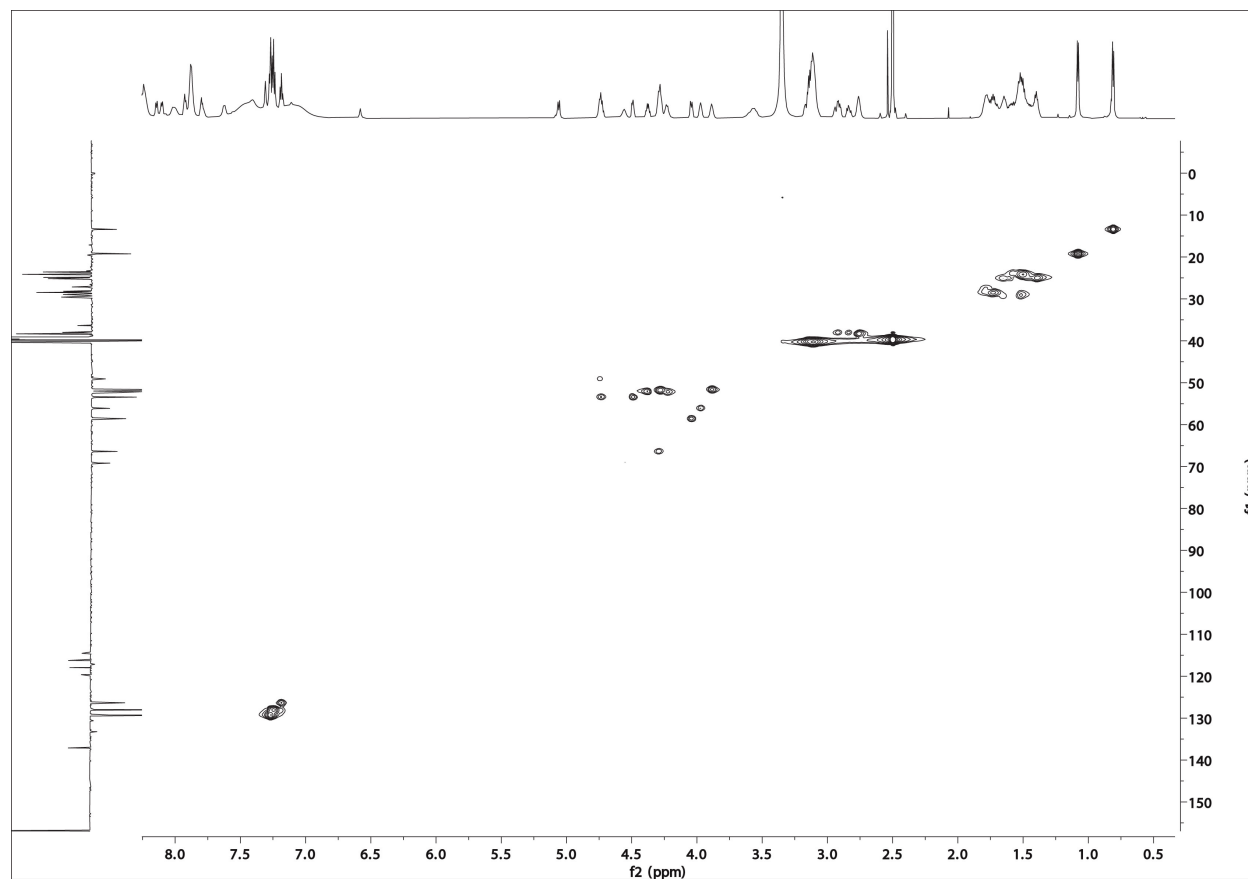
Supplementary Fig. 16 | ^1H NMR spectrum of MKM-E



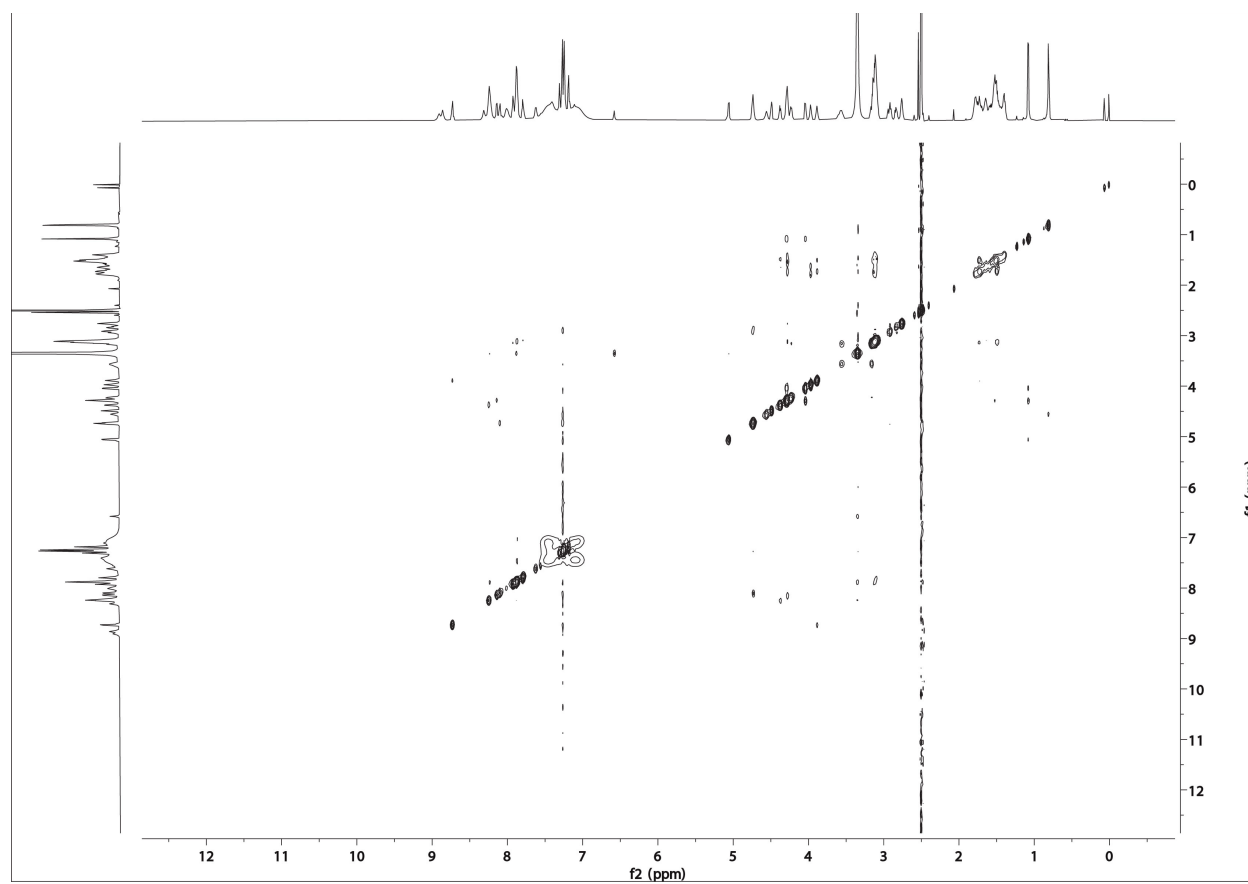
Supplementary Fig. 17 | ^{13}C DEPTQ NMR spectrum of MKM-E



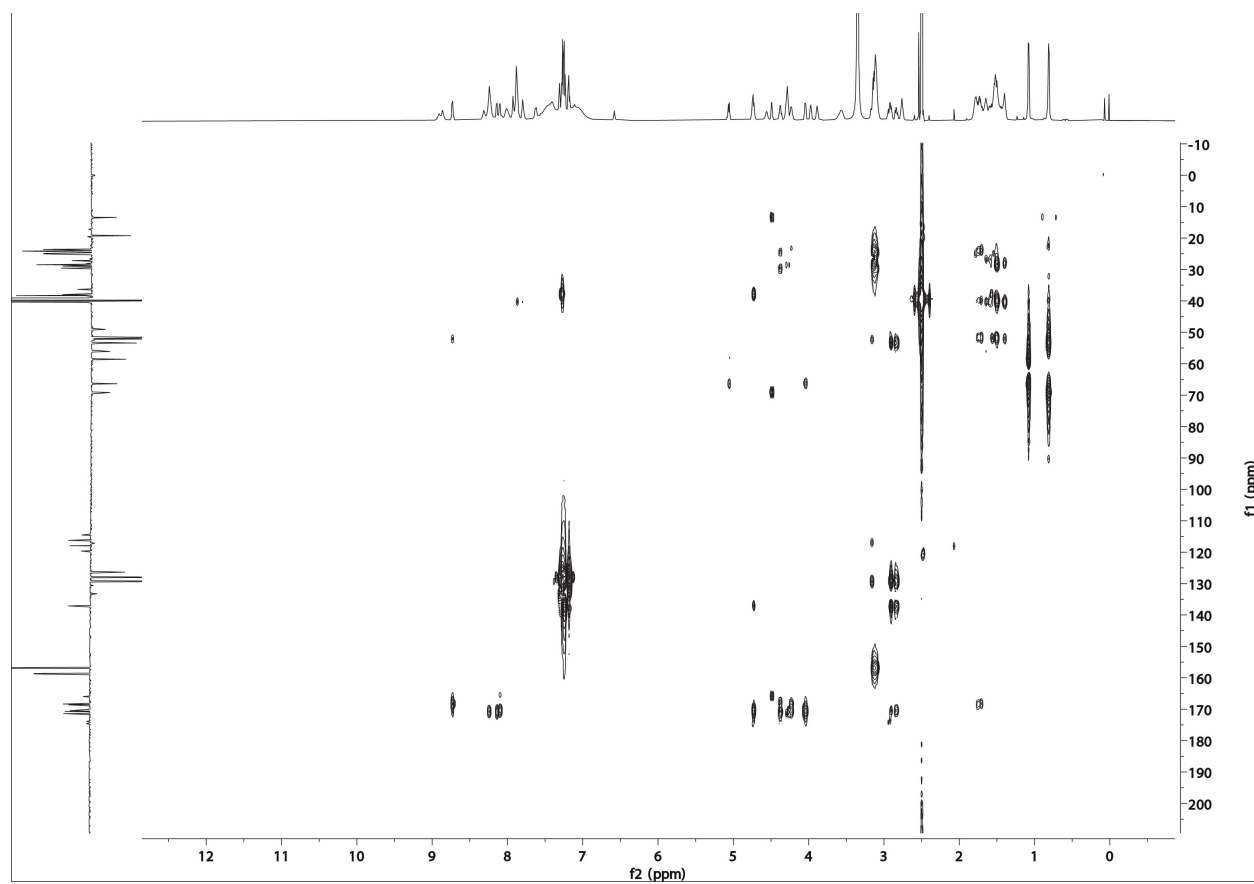
Supplementary Fig. 18 | ^1H - ^1H COSY NMR spectrum of MKM-E



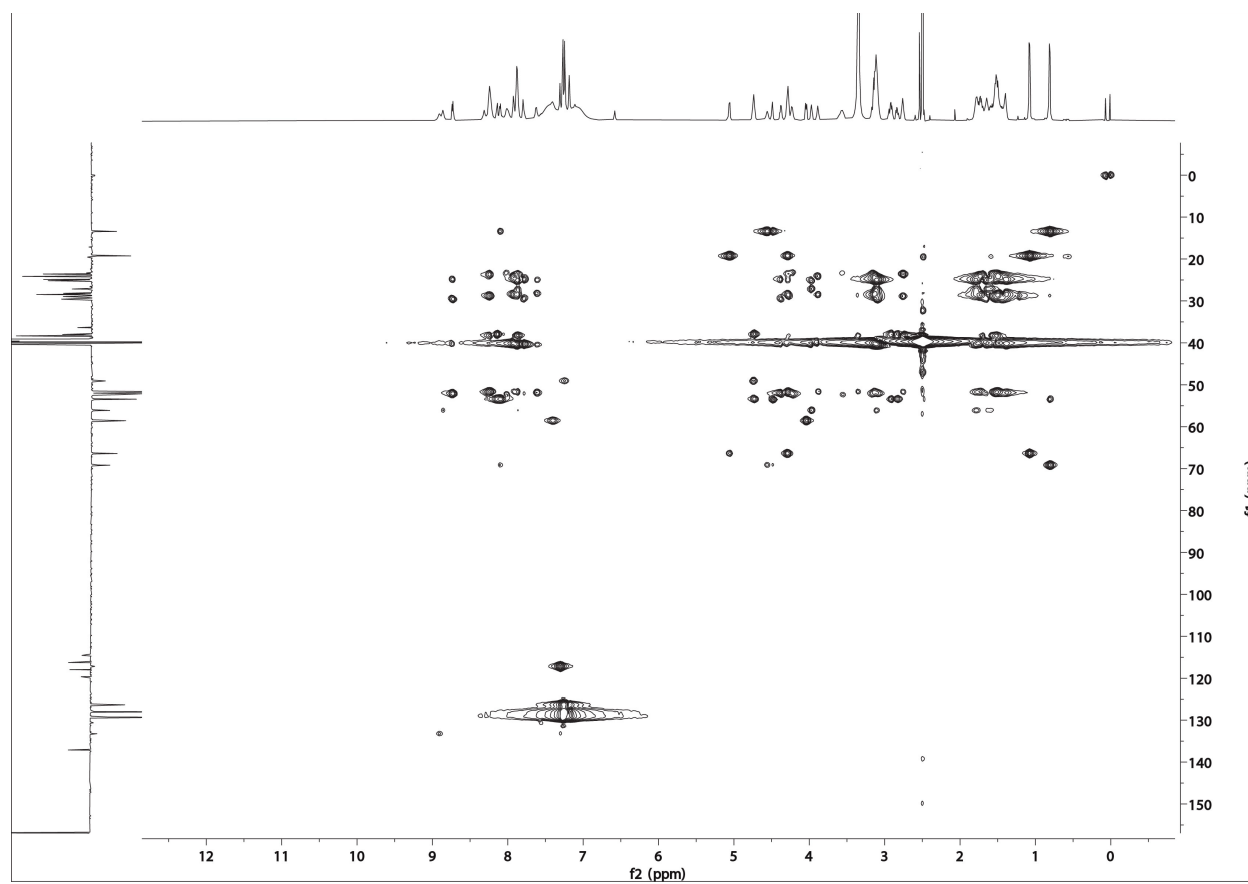
Supplementary Fig. 19 | ^1H - ^{13}C HSQC NMR spectrum of MKM-E



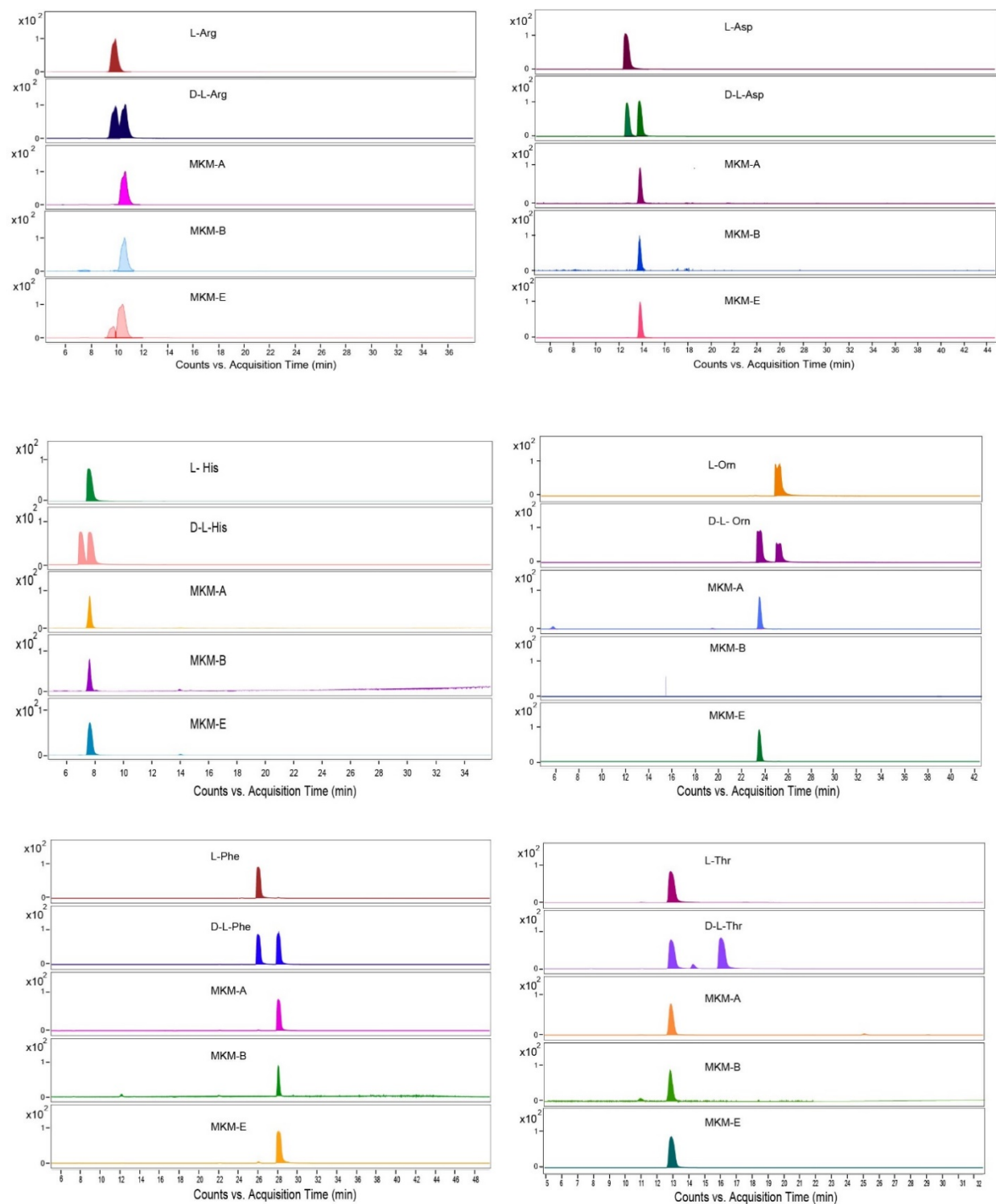
Supplementary Fig. 20 | ^1H - ^{13}C HMBC NMR spectrum of MKM-E



Supplementary Fig. 21 | TOCSY- HSQC NMR spectrum of MKM-E

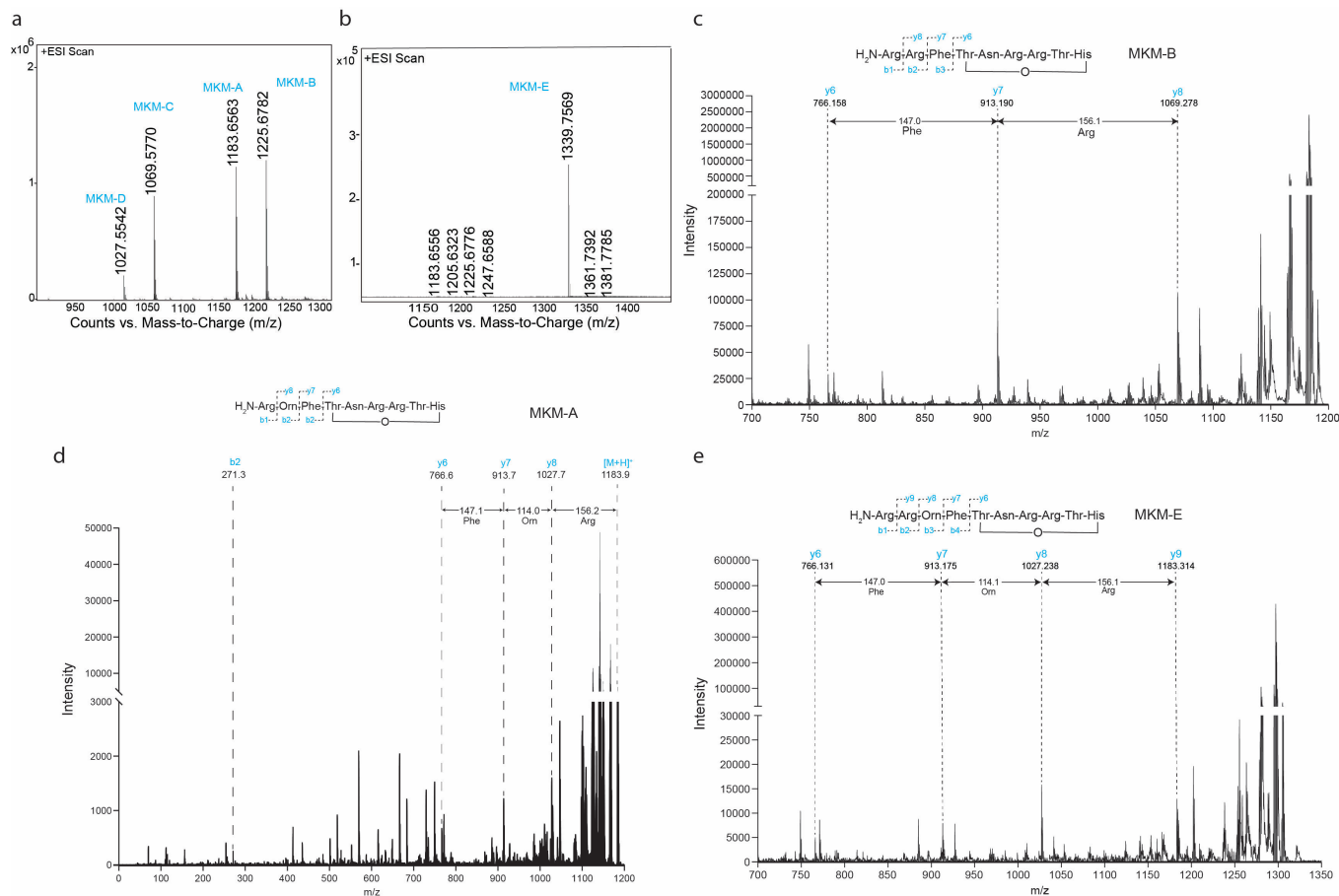


Supplementary Fig. 22 | NOESY-NMR spectrum of MKM-E

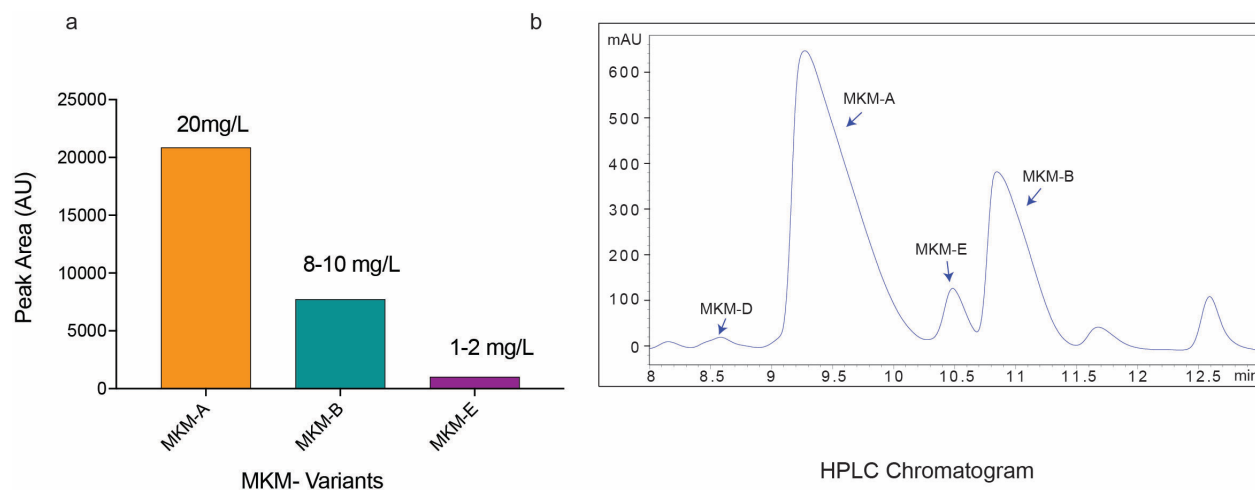


Supplementary Fig. 23 | Extracted Ion Chromatogram (EIC) of acid-hydrolyzed MKM variants treated with Marfey's reagent. Each chromatogram displays the EIC from the MKM variant samples, highlighting the peaks corresponding to the L- and D-amino acids as indicated. The retention times align with those of standard amino acids run under similar chromatographic

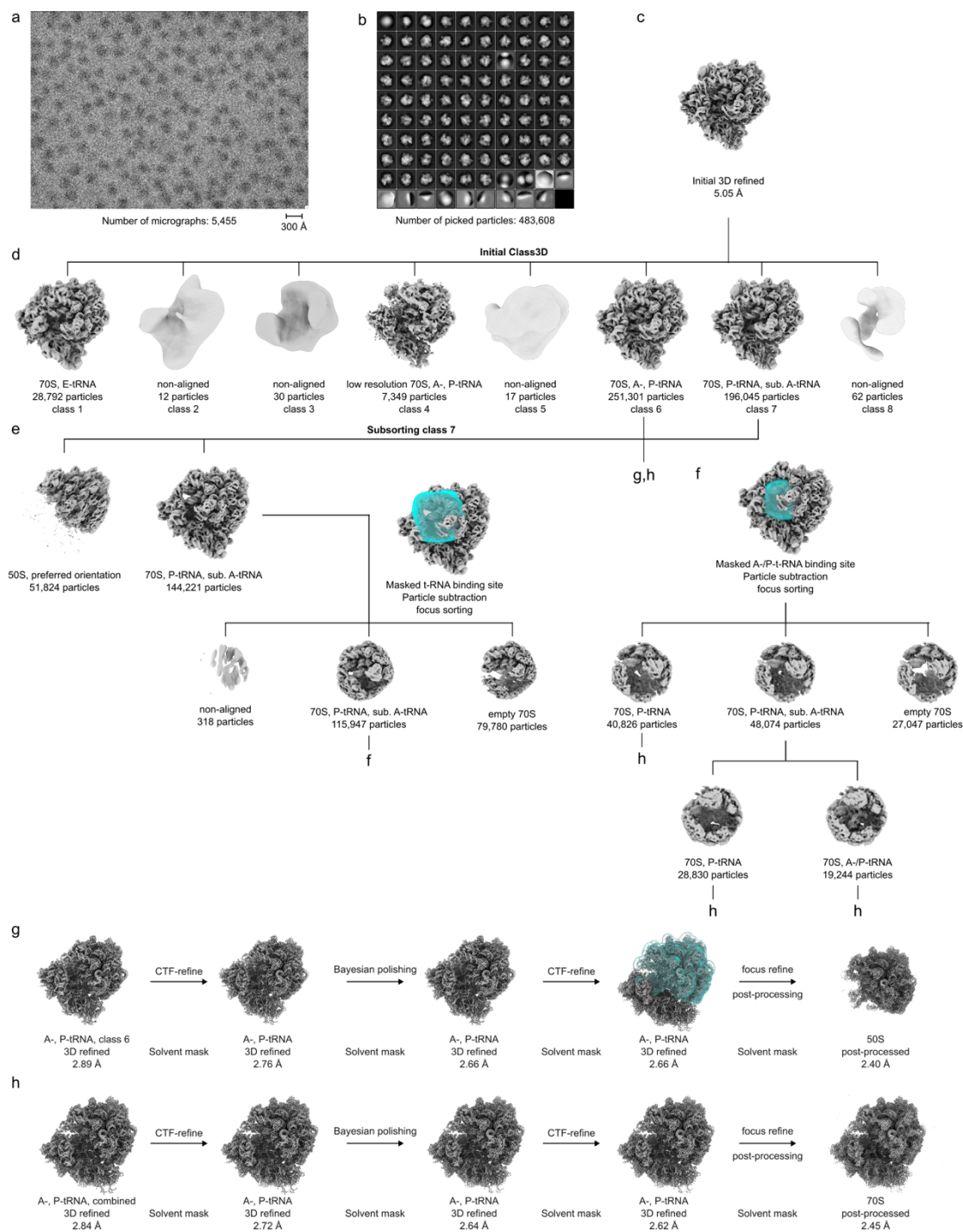
conditions. Because Asn converts to Asp during acid hydrolysis, the stereochemical comparison is made using Asp standards.



Supplementary Fig. 24 | Mass spectrometry analysis of MKM variants. a, LC-ESI-HRMS analysis of MKM-A, MKM-B, MKM-C, and MKM-D, showing the high-resolution mass spectra for each variant. **b**, LC-ESI-HRMS analysis of MKM-E. **c-e**, Tandem mass spectrometry (MALDI-MS/MS) spectrum of MKM-A, MKM-B and MKM-E. For MKM-C and MKM-D, which were available only in very low quantities, the comparative MS allowed us to propose plausible octapeptide sequences.

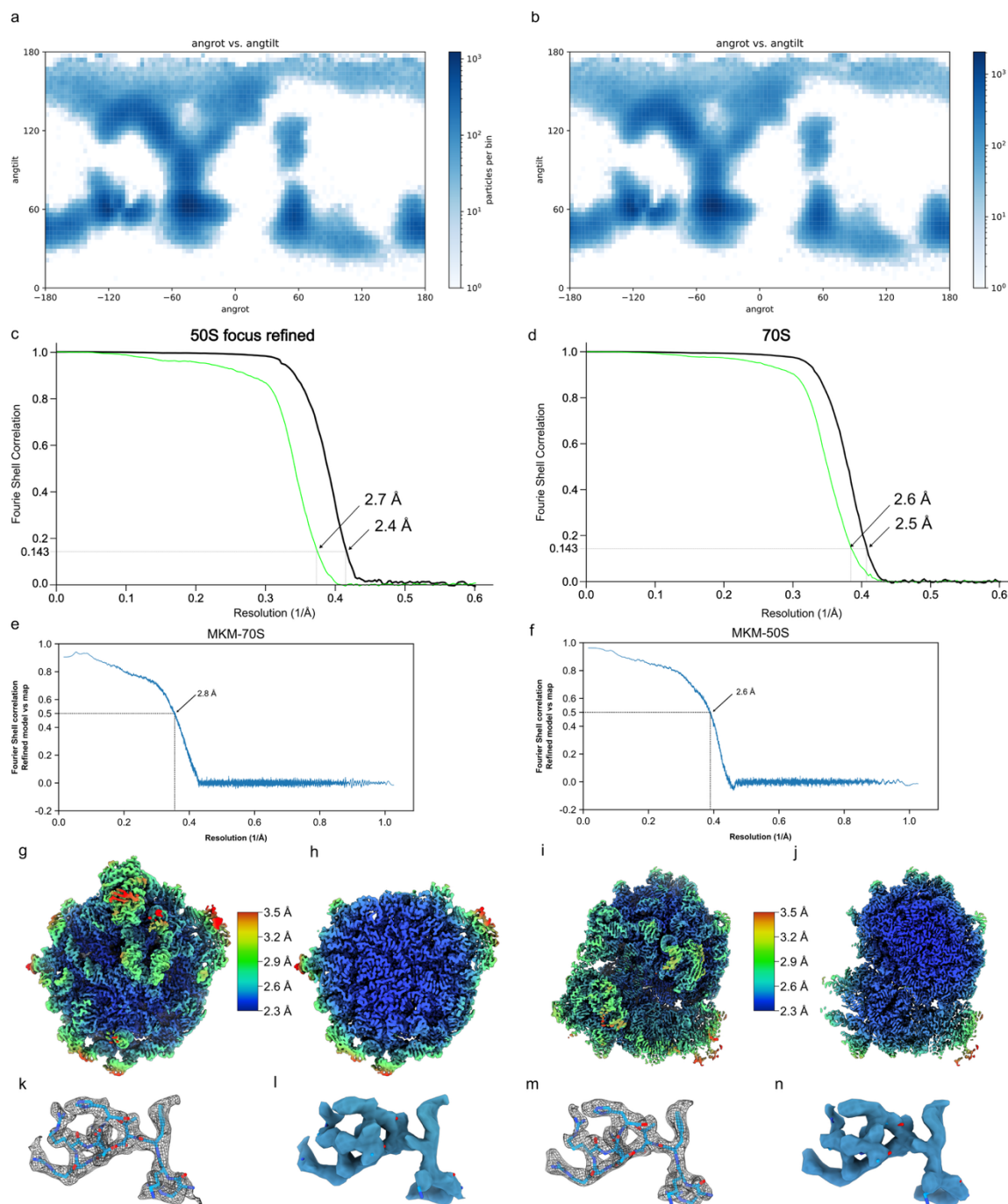


Supplementary Fig. 25 | (a) Yield proportions of the MKM variants. (b) HPLC chromatogram displaying the peaks corresponding to the individual MKM variants.



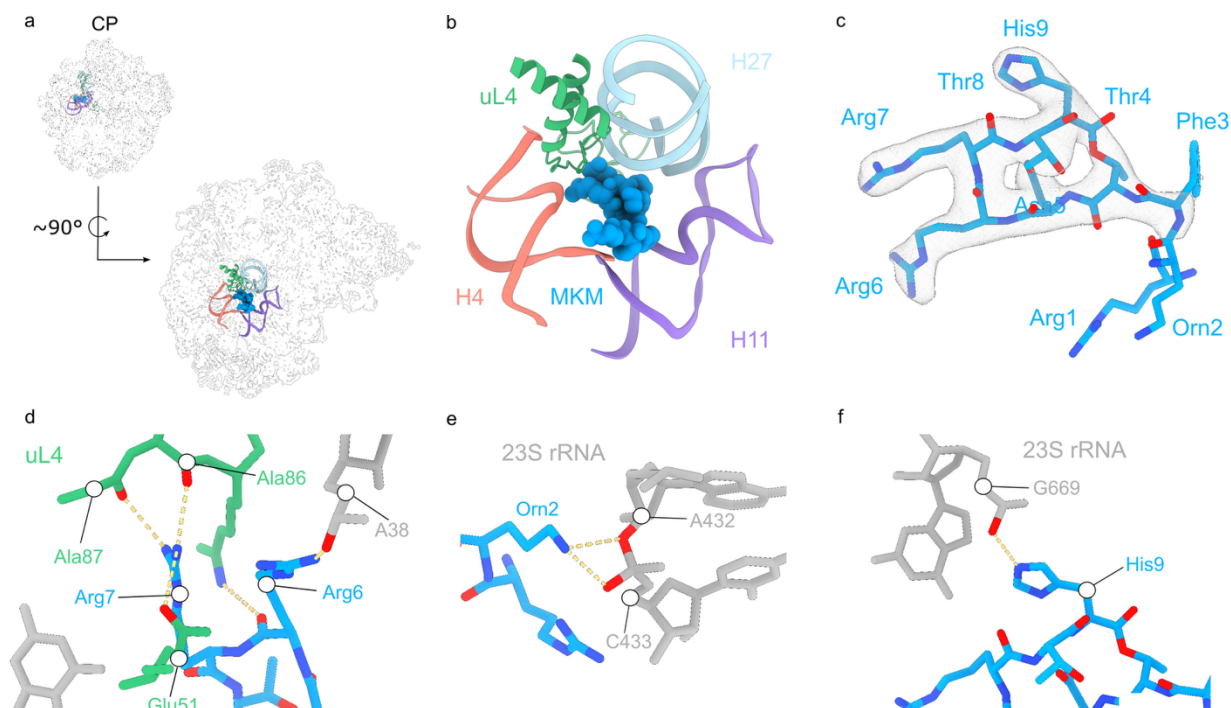
Supplementary Fig. 26. | In silico sorting scheme of the *E. coli* MKM complex. a-b, From (a), 5,455 micrographs (scale bar indicates 300 Å), (b) 483,608 ribosome-like particles were selected after 2D-classification. c, Particles were 3D refined at 4x decimated pixel size. d, Initial 3D classification for 200 iterations led to eight subclasses. e-f, The class 7 containing 70S with substoichiometric A-tRNA, was further subsorted (e) and the resulting 70S subclass was

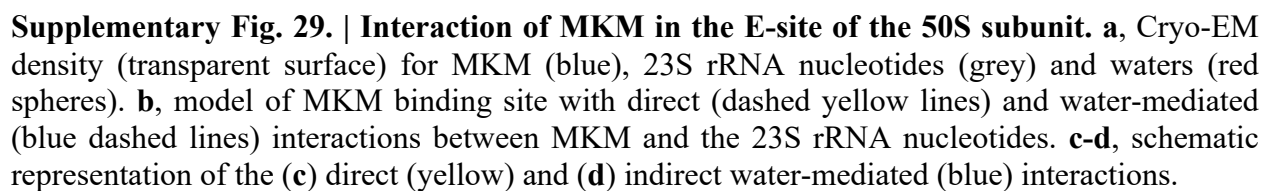
subjected to 200 iterations of focused 3D classification with (f) a mask around the tRNA binding sites. g, The 70S complex containing A-tRNA and P-tRNA density was 3D refined at undecimated pixel size and subjected to CTF refinement (4th order aberrations, beam-tilt, anisotropic magnification and per-particle defocus value estimation), Bayesian polishing and again CTF refined. 70S particles were subtracted with a mask around the 50S subunit and subsequent 3D refinement resulted in a final average resolution for the masked reconstruction of 2.4 Å (at FSC0.143). h, 70S classes containing no E-tRNA were combined, refined at undecimated pixel size, subjected to CTF refinement (4th order aberrations, beam-tilt, anisotropic magnification and per-particle defocus value estimation), Bayesian polishing, again CTF refined and after a final 3D refinement yielded a final average resolution of 2.45 Å (at FSC0.143).

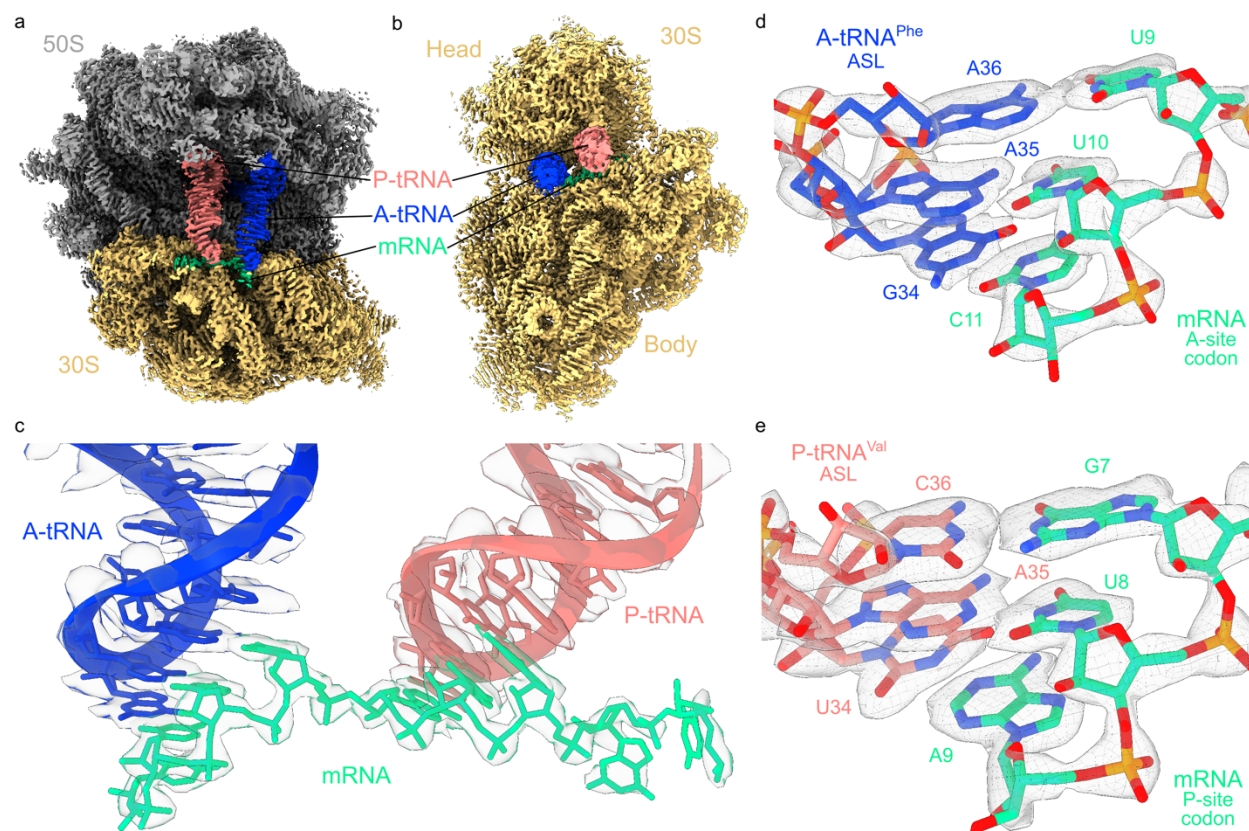


Supplementary Fig. 27. | Angular distribution and local resolution of MKM cryo-EM maps.

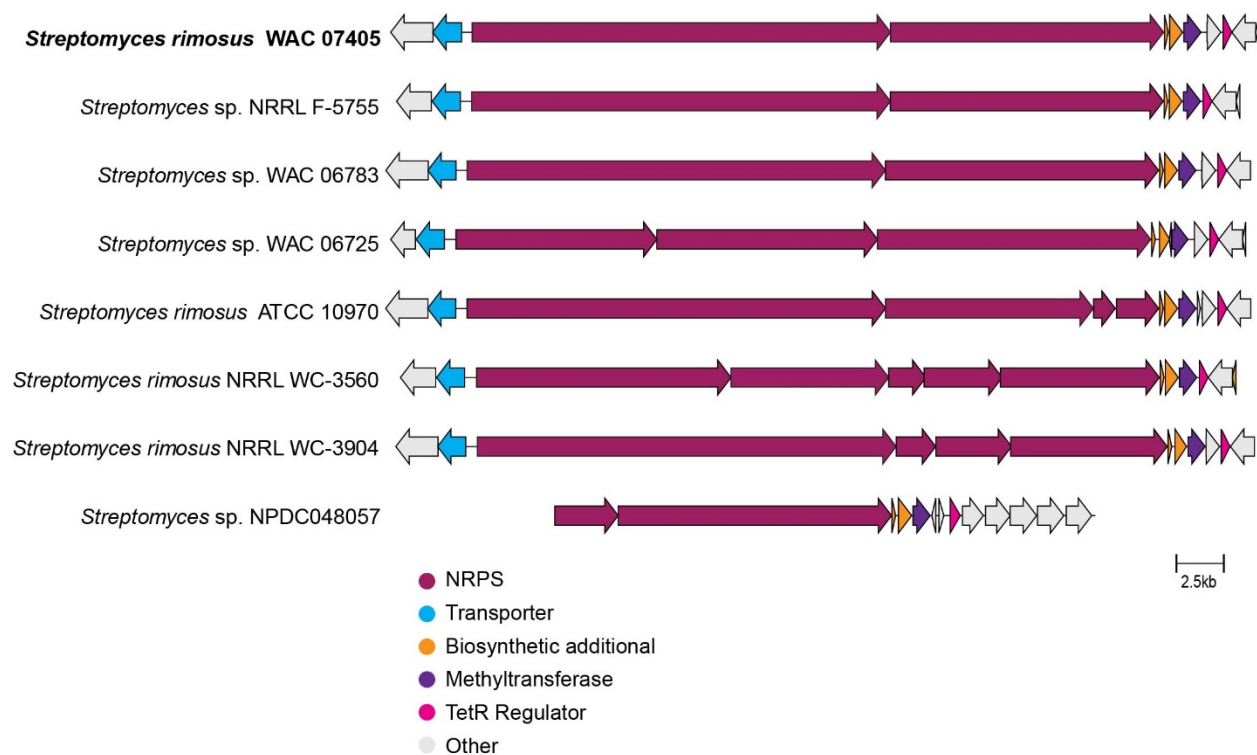
a-b, Angular distribution of the particles that comprise the (a) 50S focus refined map and (b) the combined 70S classes. **c-d**, Fourier shell correlation (FSC) curve (green, unmasked; black, masked) of the (c) 50S focus refined cryo-EM map and (d) the 70S complex. **e-f**, FSC map versus model for (e) MKM-70S and (f) MKM-50S structures. **g-j**, Cryo-EM density map colored according to local resolution for (g) the 50S subunit with (h) transverse section and (i) the 70S ribosome with (j) transverse section. **k-n**, Cryo-EM density for MKM from (k-l) the 50S subunit and (m-n) the 70S ribosome, shown as (k,m) mesh with molecular model or (l,n) coloured according to local resolution.







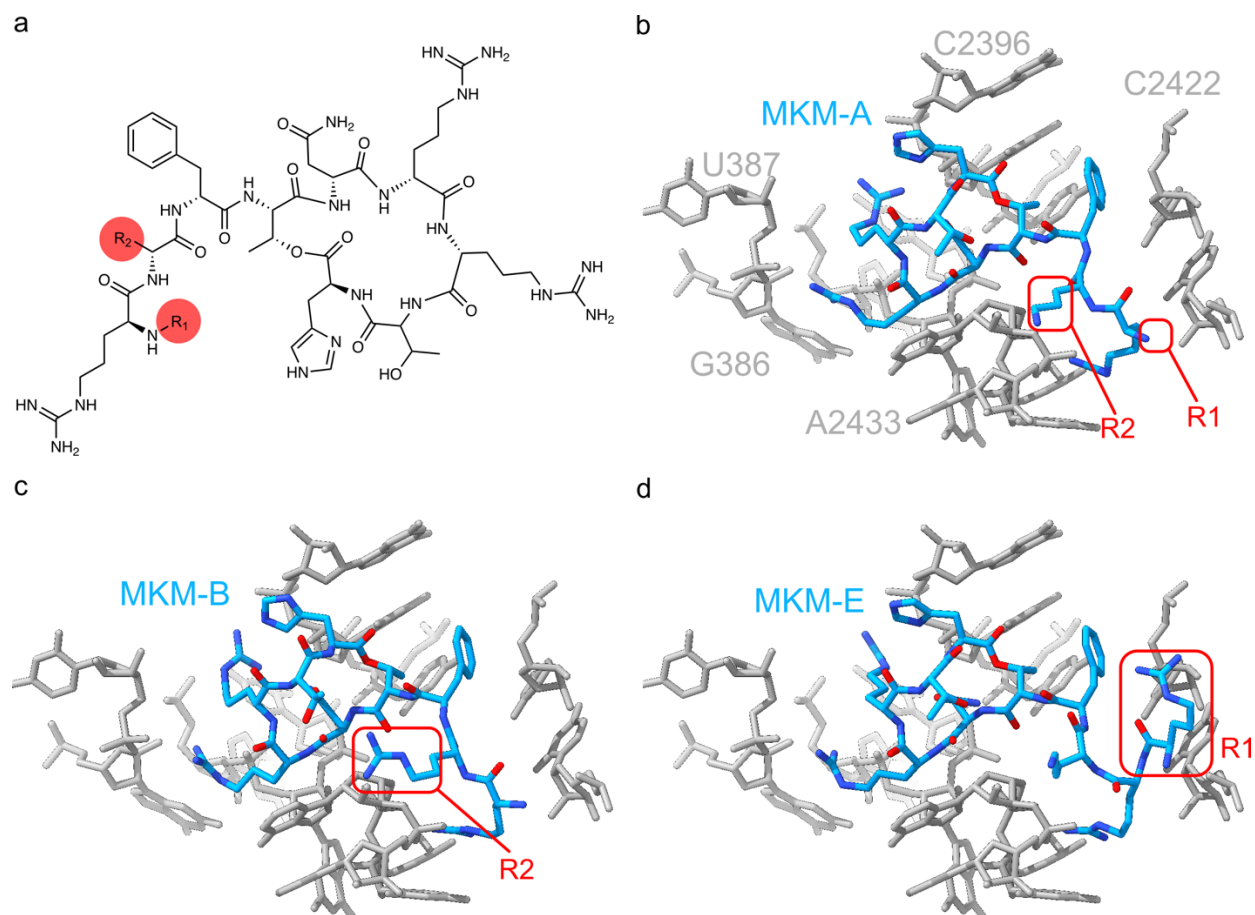
Supplementary Fig. 30 | tRNA identity and codon-anticodon interaction. a-b, cryo-EM map of the MKM-stalled 70S ribosome, shown as (a) transverse section with P-tRNA (salmon), A-tRNA (blue) and mRNA (green) highlighted and (b) 30S interface view. c, extracted cryo-EM density (grey mesh) and model of P-tRNA (salmon), A-tRNA (blue) and mRNA (green). d-e, extracted cryo-EM density of the codon-anticodon interaction for the (d) A-site tRNA^{Phe} anticodon-stem loop (blue, GAA) and A-site codon (green, UUC) and (e) P-site tRNA^{Val} anticodon-stem loop (salmon, UAC) and P-site codon (green, GUA).



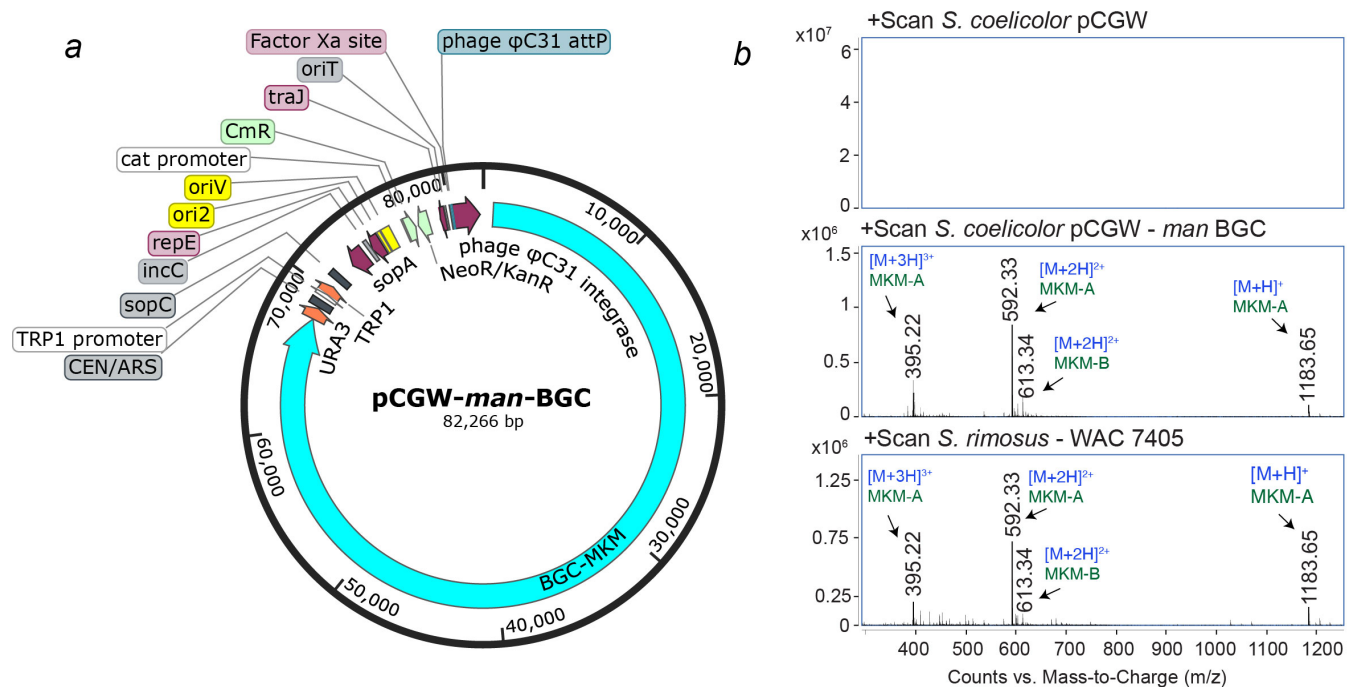
Supplementary Fig. 31 | BLAST analysis of the MKM BGC. BLAST analysis of the MKM BGC reveals its presence exclusively in the genomes of *Streptomyces rimosus*. The different genes involved in MKM biosynthesis are color-coded to distinguish them from additional associated genes, with each color representing a distinct functional group within the BGC.

Module	Stachelhaus sequence
Module 1- L-ARG	DTDDVGFVDK
Module 2- D-ARG	DSDDVGFVDK
Module 3- D- ARG/ORN	DTDDSGCVDK
Module 7- D-ARG	DTDDVGFVDK
Module 8-D-ARG	DTDDVGFVDK

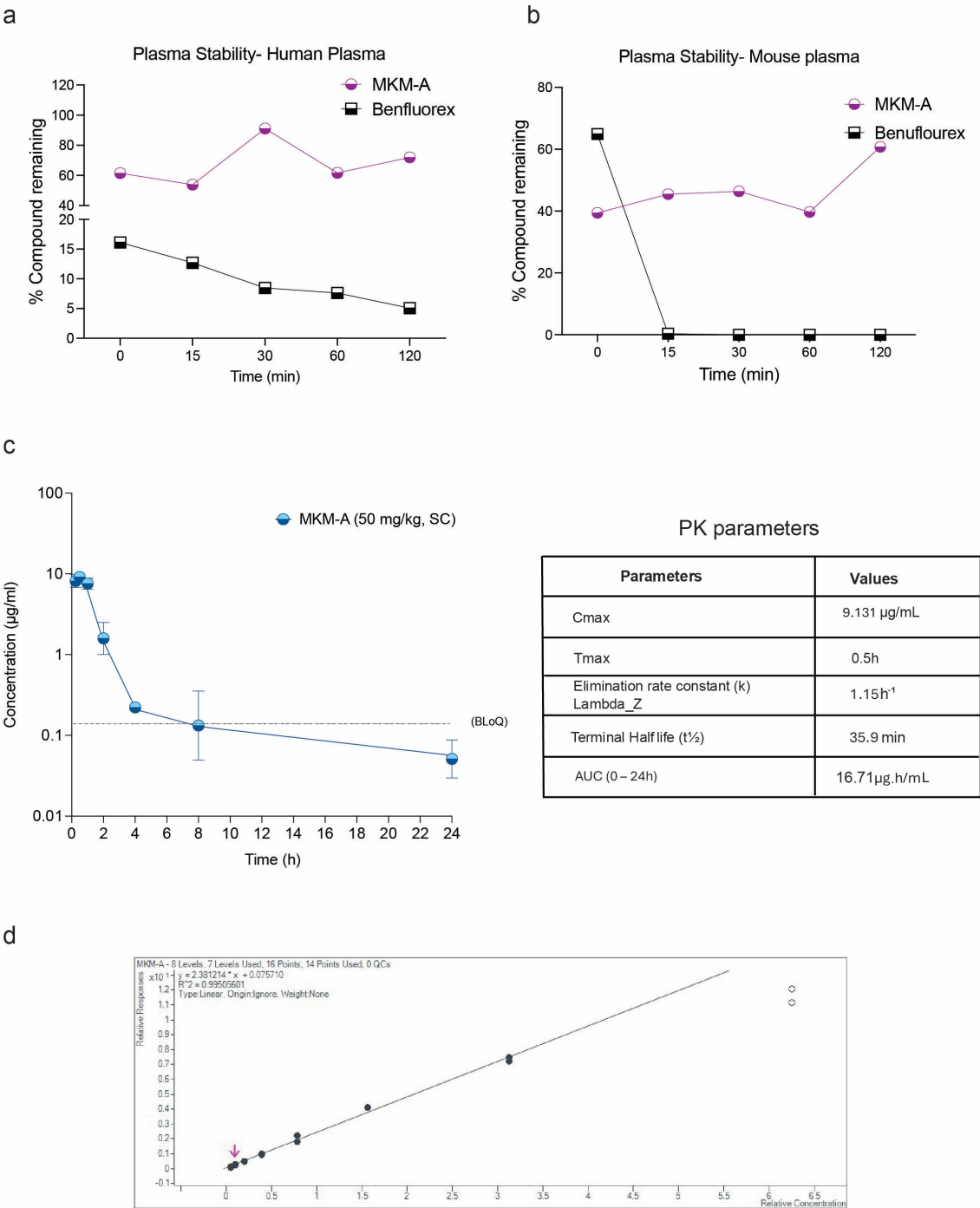
Supplementary Fig. 32 | Stachelhaus code analysis. Stachelhaus code sequences for the arginine-containing modules in the MKM BGC, generated using antiSMASH.



Supplementary Fig. 33 | Conserved binding mode of MKM congeners. Structural alignment shows that the binding mode determined for MKM-A is compatible with other MKM congeners, including MKM-B and MKM-E. This suggests that these variants likely share a common, context-specific mechanism of action to inhibit translocation, as elucidated for MKM-A.

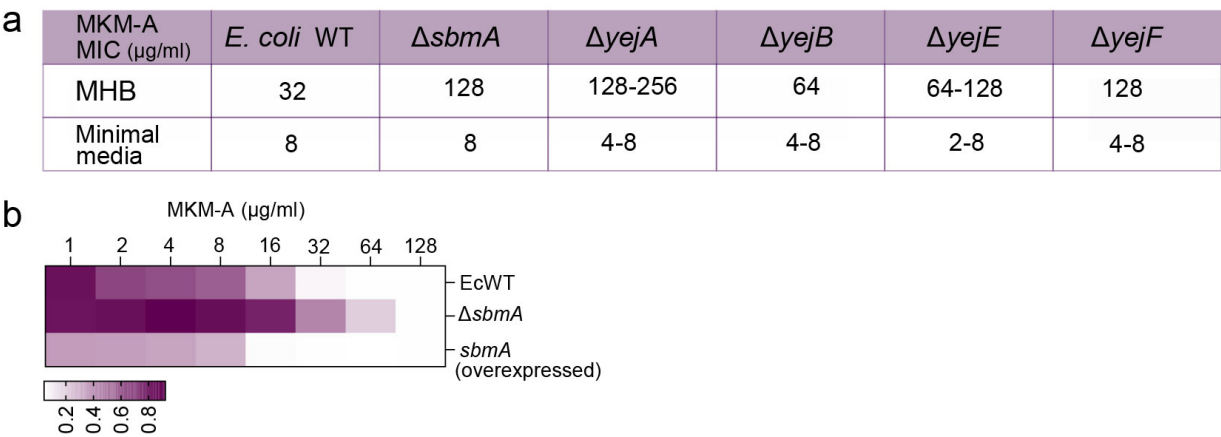


Supplementary Fig. 34 | Map of the pCGW vector with MKM BGC. (a) The pCGW plasmid served as the backbone for cloning and expression, providing essential regulatory elements. The map illustrates the successful capture and integration of the MKM BGC into the pCGW backbone, ensuring its proper organization and potential for heterologous expression. Key features of the construct, including restriction sites, selection markers, and regulatory elements, are highlighted (b) Comparison of the mass spectrometric profiles from the host strain carrying the *MKM* biosynthetic gene cluster (BGC) and the wild-type strain WAC 7405.

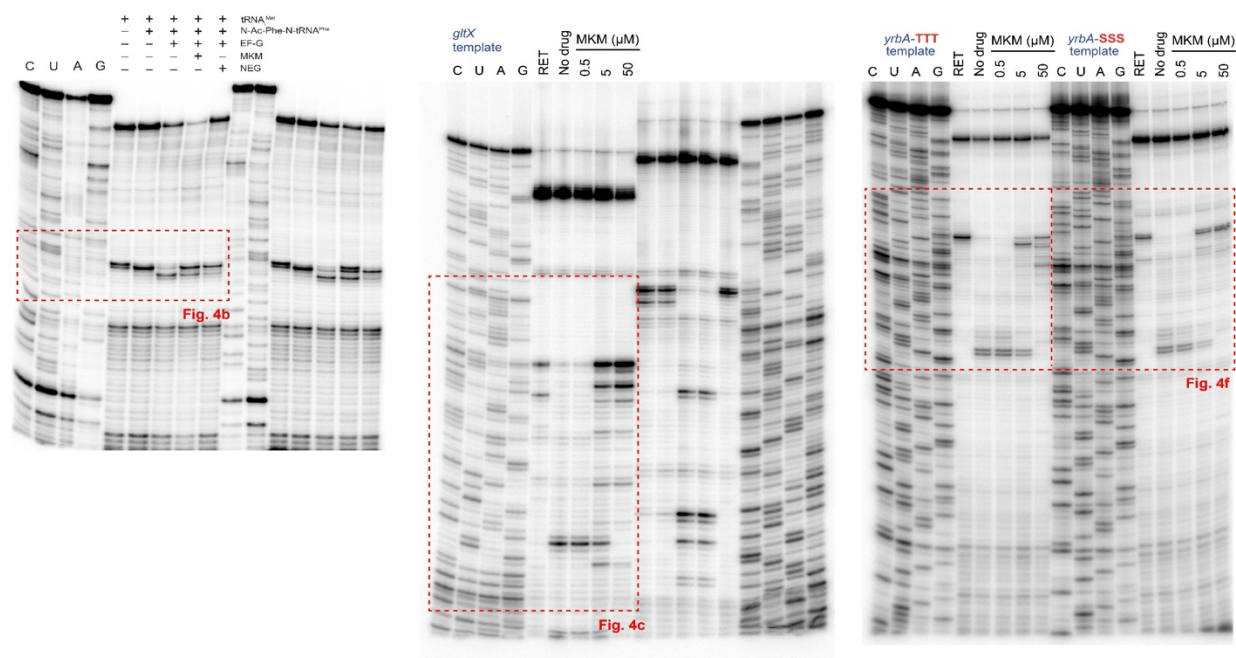


Supplementary Fig. 35| Plasma stability and pharmacokinetic characterization of MKM-

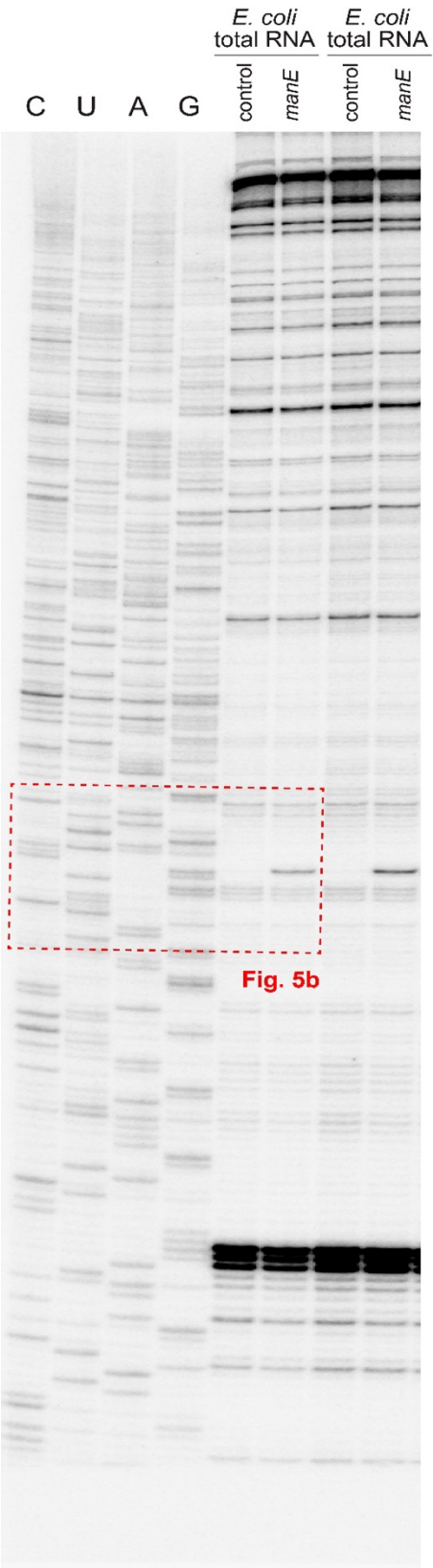
A. (a, b) Comparative plasma stability profiles of MKM-A and benfluorex in human plasma (a) and mouse plasma. Data shows mean values of two replicates and is representative of two biological repeats (b), demonstrating superior stability of MKM-A over 120 minutes. (c) Plasma concentration vs time curve following subcutaneous administration of MKM-A (50 mg/kg) in mice with corresponding pharmacokinetic parameters. The dashed line indicates the approximate Below lower limit of quantification (BLoQ). Data represent mean $\pm$ SD; n = 3 per timepoint. (d) MKM-A standards for calibration curve were prepared in a matrix of plasma at the following concentration: 100, 50, 25, 12.5, 6.25, 3.12, 1.6, and 0.8 $\mu\text{g/ml}$. 100 $\mu\text{g/ml}$ was above the linear range used for quantitation and 0.8 $\mu\text{g/ml}$ was the lower limit of detection (LLOD). Calibrants contained the same amount of internal standard as the test samples (16 $\mu\text{g/ml}$). The linear range of detection was from 1.6 to 50 $\mu\text{g/ml}$.



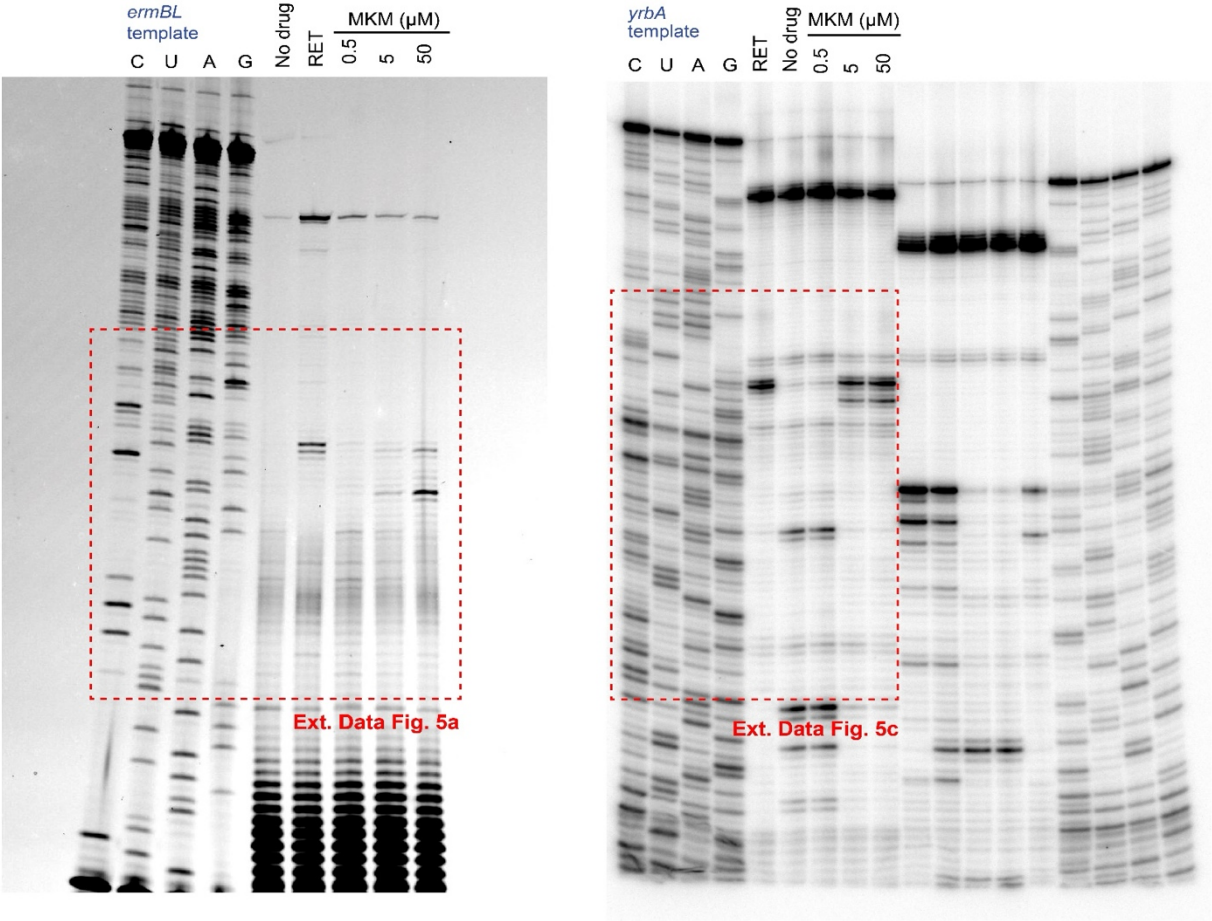
Supplementary Fig. 36 | Role of peptide uptake transporters SbmA and YejABEF in MKM susceptibility in *E. coli*. **a**, MIC of MKM for *E. coli* strains lacking SbmA, YejA, YejB, YejE, or YejF in cation-adjusted MHB medium and MOPS minimal medium. These deletion strains are part of the Keio collection¹. **b**, Comparison of MICs upon deletion or overexpression of SbmA in wild-type *E. coli*. The overexpressed strain was a part of the ASKA library². The data are displayed as a heatmap, where darker colours indicate relative growth based on OD₆₀₀ values, as shown in the scale bar.



Supplementary Fig. 37 | Raw blots for toeprinting experiments used in Fig. 4b,c,f. The red box highlights the area used in main Figure panels.



Supplementary Fig. 38 | Raw blot for toeprinting experiment used in Fig. 5b. The red box highlights the area used in main Figure panels.



Supplementary Fig. 39 | Raw blots for toeprinting experiments used in Extended data Fig. 4 a, c. The red box highlights the area used in main Figure. panels.

SUPPLEMENTARY TABLES

Supplementary Table 1 | ^1H and ^{13}C NMR assignment of MKM-A

No.	δ_{H}	δ_{C}	No.	δ_{H}	δ_{C}
Arg1 CO		168.3	Arg2 CO		171.5
α	3.79 (m)	51.7	α	3.97 (m)	56.1
β	1.65 (2H, m, overlapped)	28.4	β	1.73 (m, overlapped)	27.2
				1.80 (m, overlapped)	
γ	1.47 (2H, m, overlapped)	24.0	γ	1.66 (2H, m, overlapped)	25.1
δ	3.08 (2H, m, overlapped)	40.2	δ	3.08 (2H, m, overlapped)	40.3
$\underline{\text{C}}=\text{NH}$		156.8	$\underline{\text{C}}=\text{NH}$		156.8
$\alpha\text{-NH}$	8.21 (br s)		$\alpha\text{-NH}$	8.75 (br s)	
Ornithine CO		170.6	Arg3 CO		171.4
α	4.37 (m)	51.6	α	4.28 (m, overlapped)	51.8
β	1.71 (m, overlapped)	29.0	β	1.60 (m, overlapped)	28.0
	1.51 (m, overlapped)			1.46 (m, overlapped)	
γ	1.60 (m, overlapped)	23.4	γ	1.40 (2H, m, overlapped)	25.0
	1.56 (m, overlapped)				
δ	2.78 (2H, m, overlapped)	38.3	δ	3.08 (2H, m, overlapped)	40.3
$\alpha\text{-NH}$	8.56 (d, $J = 5.0$ Hz)		$\underline{\text{C}}=\text{NH}$		156.8
NH2	7.81 (brs, overlapped)		$\alpha\text{-NH}$	7.60 (d, $J = 9.0$ Hz)	
Phe CO		170.3	Thr2 CO		170.5
α	4.74 (m, overlapped)	53.4	α	4.07 (br d, $J = 8.7$ Hz)	58.6
β	2.92 (dd, $J = 13.4, 6.8$ Hz)	38.1	β	4.30 (m, overlapped)	66.4
	2.82 (dd, $J = 13.4, 8.6$ Hz)				
1'		137.1	γ	1.08 (d, $J = 6.5$ Hz)	19.2
2', 6'	7.26 (t, $J = 7.3$ Hz)	129.2	$\alpha\text{-NH}$	7.39 (br d, $J = 8.7$ Hz)	
3', 5'	7.25 (t, $J = 7.2$ Hz)	128.0	OH	5.07 (br d, $J = 8.3$ Hz)	
4'	7.19 (t, $J = 7.3$ Hz)	126.3	His CO		168.5
$\alpha\text{-NH}$	8.25 (d, $J = 7.2$ Hz)		α	4.11 (br dd, $J = 5.5, 14.7$ Hz)	52.4
Thr1 CO		166.0	β	3.54 (m, overlapped)	23.4
				3.19 (dd, $J = 5.5, 15.3$ Hz)	
α	4.50 (dd, $J = 4.1, 7.1$ Hz)	53.5	1'		129.4
β	4.57 (dq, $J = 7.1, 6.2$ Hz)	69.1	2'	7.30 (br s)	116.9
γ	0.82 (d, $J = 6.2$ Hz)	13.4	4'	8.94 (br s)	133.2
$\alpha\text{-NH}$	8.15 (d, $J = 4.1$ Hz)		$\alpha\text{-NH}$	8.04 (br s)	
Asn CO		174.6	3'-NH	8.94 (br s)	
α	4.76 (m, overlapped)	49.0			
β	3.50 (m, overlapped)	36.3			
	2.94 (m, overlapped)				
$\underline{\text{COONH}}_4$		173.8			
$\alpha\text{-NH}$	7.25 (m, overlapped)				

Supplementary Table 2 | ^1H and ^{13}C NMR assignment of MKM-B

No.	δ_{H}	δ_{C}	No.	δ_{H}	δ_{C}
Arg1 CO		168.3	<u>COONH₄</u>		174.6
α	3.78 (m)	51.7	α -NH	7.25 (m, overlapped)	
β	1.65 (2H, m, overlapped)	28.4	Arg6 CO		171.5
γ	1.48 (2H, m, overlapped)	24.0	α	3.97 (m)	56.1
δ	3.08 (2H, m, overlapped)	40.1	β	1.72 (m, overlapped)	27.2
				1.82 (m, overlapped)	
<u>C=NH</u>		156.9	γ	1.65 (m, overlapped)	25.2
				1.59 (m, overlapped)	
α -NH	8.19 (m, overlapped)		δ	3.08 (2H, m, overlapped)	40.4
Arg2 CO		170.6	<u>C=NH</u>		156.9
α	4.33 (m, overlapped)	52.3	α -NH	8.75 (br s)	
β	1.67 (m, overlapped)	29.4	Arg7 CO		171.5
	1.47 (m, overlapped)				
γ	1.65 (m, overlapped)	25.1	α	4.29 (m, overlapped)	51.8
	1.41 (m, overlapped)				
δ	3.08 (2H, m, overlapped)	40.3	β	1.65 (m, overlapped)	28.1
				1.42 (m, overlapped)	
α -NH	8.55 (d, $J = 7.6$ Hz)		γ	1.43 (2H, m, overlapped)	25.0
			δ	3.08 (2H, m, overlapped)	40.3
Phe3 CO		170.4	<u>C=NH</u>		156.9
α	4.74 (m, overlapped)	53.4	α -NH	7.59 (d, $J = 8.7$ Hz)	
β	2.92 (dd, $J = 13.5, 5.8$ Hz)	38.0	Thr8 CO		170.8
	2.82 (dd, $J = 13.5, 9.0$ Hz)				
1'		137.1	α	4.08 (m, overlapped)	58.6
2', 6'	7.28 (d, $J = 7.5$ Hz)	129.2	β	4.29 (m, overlapped)	66.4
3', 5'	7.24 (t, $J = 7.2$ Hz)	128.0	γ	1.08 (d, $J = 6.4$ Hz)	19.2
4'	7.18 (t, $J = 7.1$ Hz)	126.4	α -NH	7.41 (overlapped)	
α -NH	8.20 (m, overlapped)		OH	5.09 (br d, $J = 8.0$ Hz)	
Thr4 CO		166.0	His9 CO		168.5
α	4.50 (dd, $J = 4.3, 7.1$ Hz)	53.5	α	4.06 (m, overlapped)	52.4
β	4.58 (dd, $J = 4.3, 4.8$ Hz)	69.2	β	3.54 (m, overlapped)	23.2
				3.19 (dd, $J = 4.7, 15.1$ Hz)	
γ	0.82 (d, $J = 6.3$ Hz)	13.4	1'		129.4
α -NH	8.13 (d, $J = 7.1$ Hz)		2'	7.29 (br s)	116.9
Asn CO		173.8	4'	8.93 (br s)	133.3
α	4.77 (m, overlapped)	49.0	α -NH	8.04 (br s)	
β	3.49 (m, overlapped)	36.2			
	2.93 (m, overlapped)				

Supplementary Table 3 | ^1H and ^{13}C NMR assignment of MKM-E

No.	δ_{H}	δ_{C}	No.	δ_{H}	δ_{C}
Arg1 CO		168.3	α -NH	8.10 (d, $J = 7.1$ Hz)	
α	3.89 (m)	51.7	Asn6 CO		173.9
β	1.73 (2H, m, overlapped)	28.5	α	4.74 (m, overlapped)	49.1
γ	1.50 (2H, m, overlapped)	24.2	β	3.58 (m, overlapped)	36.3
δ	3.08 (2H, m, overlapped)	40.0		2.93 (m, overlapped)	
$\text{C}=\text{NH}$		156.9	COONH		174.6
α -NH ₂	8.23 (m)		α -NH	7.25 (m, overlapped)	
Arg2 CO		170.7	Arg7 CO		171.5
α	4.38 (m)	52.1	α	3.97 (m)	56.1
β	1.66 (m, overlapped)	29.6	β	1.78 (2H, m, overlapped)	27.2
	1.52 (m, overlapped)		γ	51.65 (2H, m, overlapped)	25.2
γ	1.43 (2H, m, overlapped)	24.9	δ	3.11 (2H, m, overlapped)	40.3
δ	3.11 (2H, m, overlapped)	40.0	$\text{C}=\text{NH}$		156.9
$\text{C}=\text{NH}$		156.9	α -NH	8.86 (br s)	
α -NH	8.73 (br s)		Arg8 CO		171.4
Ornithine3 CO		171.0	α	4.28 (m, overlapped)	51.9
α	4.27 (m)	51.7	β	1.78 (m, overlapped)	28.2
β	1.73 (m, overlapped)	28.9		1.51 (m, overlapped)	
	1.53 (m, overlapped)		γ	1.65 (m, overlapped)	25.0
γ	1.58 (m, overlapped)	23.6		1.40 (m, overlapped)	
	1.50 (m, overlapped)		δ	3.11 (2H, m, overlapped)	40.3
δ	2.76 (2H, m, overlapped)	38.3	$\text{C}=\text{NH}$		156.9
α -NH	8.25 (m, overlapped)		α -NH	7.62 (d, $J = 8.5$ Hz)	
NH ₂	7.81 (m, overlapped)		Thr9 CO		170.5
Phe4 CO		170.3	α	4.04 (dd, $J = 8.7, 2.1$ Hz)	58.6
α	4.73 (m, overlapped)	53.4	β	4.29 (m, overlapped)	66.4

β	2.92 (dd, $J = 13.7, 4.6$ Hz) 2.83 (dd, $J = 13.7, 8.4$ Hz)	38.0	γ	1.08 (d, $J = 6.4$ Hz)	19.3
1'		137.2	α -NH	7.41 (br s)	
2', 6'	7.26 (d, $J = 7.3$ Hz)	129.2	OH	5.06 (br d, $J = 8.2$ Hz)	
3', 5'	7.25 (t, $J = 7.3$ Hz)	128.0	His10 CO		168.3
4'	7.19 (t, $J = 7.3$ Hz)	126.4	α	4.23 (m)	52.4
α -NH	8.14 (d, $J = 7.7$ Hz)		β	3.57 (m, overlapped) 3.16 (m, overlapped)	23.3
Thr5 CO		166.0	1'		129.3
α	4.49 (dd, $J = 3.9, 7.1$ Hz)	53.5	2'	7.31 (br s)	117.1
β	4.56 (br s)	69.2	4'	8.91 (br s)	133.2
γ	0.81 (d, $J = 6.5$ Hz)	13.4	α -NH	8.01 (br s)	

Supplementary Table 4 | Calculated and Observed Masses of MKM variants.

MKMs	Chemical formula	Calculated mass [M+H] ⁺	Observed Mass [M+H] ⁺	Mass error Δ (ppm)
MKM-A	C ₅₀ H ₈₂ N ₂₂ O ₁₂	1183.6561	1183.6563	0.1
MKM-B	C ₅₁ H ₈₄ N ₂₄ O ₁₂	1225.6779	1225.6782	0.2
MKM-C	C ₄₅ H ₇₂ N ₂₀ O ₁₁	1069.5768	1069.5770	0.1
MKM-D	C ₄₄ H ₇₀ N ₁₈ O ₁₁	1027.5550	1027.5542	0.7
MKM-E	C ₅₆ H ₉₄ N ₂₅ O ₁₃	1339.7572	1339.7569	0.2

Supplementary Table 5 | Proposed functions of proteins encoded by the MKM BGC (accession no: JBPBLP010000002.1) using antiSMASH v6.0.0³ and antiSMASH v8.0.0⁴

Location	Gene	Homolog	Proposed function
ACRVTS_10640	<i>orf-2</i>	Alpha/beta hydrolase fold protein	Putative peptidase
ACRVTS_10650	<i>orf-1</i>	Penicillin amidase	Penicillin amidohydrolase
ACRVTS_10655	<i>manD</i>	ABC transporter	Transporter
ACRVTS_10660	<i>manA</i>	NRPS (modules 1-6)	Peptide synthase
ACRVTS_10665	<i>manB</i>	NRPS (modules 7-10)	Peptide synthase
ACRVTS_10670	<i>manI</i>	MbtH	MbtH-like protein
ACRVTS_10675	<i>manC</i>	Thioesterase	Thioesterase domain
ACRVTS_10680	<i>manE</i>	FtsJ-like methyltransferase	Ribosomal RNA large subunit methyltransferase
ACRVTS_10690	<i>man2</i>	(NADP oxidoreductase coenzyme F420-dependent)	Oxidoreductase
ACRVTS_10695	<i>manR</i>	Bacterial regulatory proteins, TetR family	Transcriptional regulator
ACRVTS_10700	<i>man3</i>	Amidase [<i>Pseudomonas putida</i>]	Amidase activity

Supplementary Table 6 | Mutations in MKM-resistant *E. coli* mutants from serial passaging

Mutant ID	Mutation/position	Gene(s)	Function	Gene_synonym	locus_tag
Ecmut1	+G(675/1221 nt)	<i>sbmA</i>	nonspecific peptide importer of the inner membrane	ECK0372; JW0368	BW25113_0377
Ecmut1	Δ1bp (1900/2598 nt)	<i>topA</i>	DNA topoisomerase I	ECK1268; JW1266	BW25113_1274
Ecmut1	Δ208 bp	[<i>rpmI</i>]	[<i>rpmI</i>]	ECK1715; JW1707	BW25113_1717
Ecmut2	Δ5,466 bp (IS3-mediated)	<i>yaiT</i> – [<i>yaiW</i>]	<i>yaiT</i> , <i>yaiV</i> , <i>ampH</i> , <i>sbmA</i> , [<i>yaiW</i>]	ECK02368; JW0362	BW25113_4580
Ecmut2	C→TA180V (GCG→GTG)	<i>ndh</i>	respiratory NADH dehydrogenase 2 / cupric reductase	ECK1095; JW1095	BW25113_1109
Ecmut2	(T)7→6(43/198 nt)	<i>rpmI</i>	50S ribosomal subunit protein bL35	ECK1715; JW1707	BW25113_1717

Supplementary Table 7 | Bacterial strains and plasmids used in the study.

Strain	Res	Experiments/ Description	Reference or source
<i>Acinetobacter baumannii</i> ATCC17978	-	MIC determination	Wright lab strain collection
<i>Bacillus subtilis</i> 168	-	MIC determination, trpC2, wild type	Common laboratory strain
<i>Bacteroides fragilis</i> GC73	-	MIC determination, gut isolate	Surette lab collection
<i>Bifidobacterium breve</i> GC96	-	MIC determination, gut isolate	Surette lab collection
<i>Blautia obeum</i> GC925	-	MIC determination, gut isolate	Surette lab collection
<i>Candida albicans</i> ATCC 90028	-	MIC determination	Wright lab strain collection
<i>Clostridium perfringens</i> GC1584	-	MIC determination, gut isolate	Surette lab collection
<i>Escherichia coli</i> BW25113	-	MIC determination and other experiments, Wild type	Common laboratory strain
<i>E. coli</i> BW25113 Δ bamB Δ tolC	-	MIC determination and expression of resistance genes	Wright lab strain collection
<i>E. coli</i> C0617	-	MIC determination, MDR	Wright clinical collection
<i>E. coli</i> C1507	-	MIC determination, MDR	Wright clinical collection
<i>E. coli</i> C1508	-	MIC determination, MDR	Wright clinical collection
<i>E. coli</i> ET12567	-	Conjugation experiment; <i>dam-13::Tn9 dcm-6 hsdMRS cat</i>	Wright lab strain collection
<i>E. coli</i> EPI300		Inducible <i>trfA</i> controlling oriV	Wright lab strain collection
<i>E. coli</i> ET12567 pR9406	Ap	Conjugation experiment; helper strain	Wright lab strain collection
<i>E. coli</i> SQ110 Δ tolC ::pZsbaA	Ap, Sp	Selection of mutants in rRNA genes, contains a single <i>rrnE</i> operon	Mankin lab collection
<i>E. coli</i> BL21 Δ tolC		Ribosome profiling	Mankin lab collection
<i>E. coli</i> BW25113 Δ sbaA	Km	MIC determination	Keio collection
<i>E. coli</i> BW25113 Δ yejA	Km	MIC determination	Keio collection
<i>E. coli</i> BW25113 Δ yejB	Km	MIC determination	Keio collection
<i>E. coli</i> BW25113 Δ yejE	Km	MIC determination	Keio collection
<i>E. coli</i> BW25113 Δ yejF	Km	MIC determination	Keio collection
<i>E. coli</i> BW25113 Δ rpmI	Km	MIC determination	Keio collection
<i>Klebsiella pneumoniae</i> C1459	-	MIC determination, MDR	Wright clinical collection
<i>K. pneumoniae</i> C1557	-	MIC determination, MDR	Wright clinical collection
<i>K. pneumoniae</i> C1559	-	MIC determination, MDR	Wright clinical collection
<i>Lactobacillus plantarum</i> GC4	-	MIC determination, gut isolate	Surette lab collection
<i>Mycobacterium tuberculosis</i> Ra	-	MIC determination	Wright lab strain collection
<i>M. smegmatis</i> MC ² 155	-	MIC determination	Wright lab strain collection
<i>M. avium</i> subsp. <i>avium</i> ATCC 25291	-	MIC determination	Wright lab strain collection
<i>M. fortuitum</i> subsp. <i>fortuitum</i> ATCC 6841	-	MIC determination	Wright lab strain collection
<i>M. abscessus</i> ATCC 19977	-	MIC determination	Wright lab strain collection
<i>M. bovis</i> BCG Pasteur ATCC 35734	-	MIC determination	Wright lab strain collection
<i>Pseudomonas aeruginosa</i> PAO1	-	MIC determination	Wright lab strain collection

<i>Streptomyces coelicolor</i> M1154	-	Conjugation experiment	Wright lab strain collection
<i>S. aureus</i> USA 300	-	MIC determination	Wright clinical collection
<i>S. aureus</i> ATCC 29213	-	MIC determination	Wright clinical collection
<i>S. cerevisiae</i> VL6-48N		MAT α , <i>his3-D200</i> , <i>trp1-Δ1</i> , <i>ura3-Δ1</i> , <i>lys2</i> , <i>ade2-101</i> , <i>met14</i> , <i>psi+cir^O</i> , TAR host strain	Wright lab strain collection
<i>S. coelicolor</i> M1154 -MKM-BGC	Km	Heterologous expression of MKM; contains pCGW- <i>man</i> plasmid	This study
<i>S. coelicolor</i> M1154 pCGW	Km	Control strain, contain vector only	This study
<i>Streptomyces rimosus</i> WAC 7405		MKM Producer Strain	Wright Actinomycete collection
Plasmids			
pCGW	Km	Engineered copy number controlled capturing vector for TAR, <i>aac(3)IV ura3 cen/ARS trp1 sopABC repE ori2 oriV cat neo traJ-oriT attP-intϕC31</i>	Lab stock
pGDP3	Ap	a low copy plasmid with a P _{lac} promoter ⁵	Lab stock

Res. – resistance marker, MDR – multidrug resistant, Ap – ampicillin, Km – kanamycin, Sp - spectinomycin

Supplementary Table 8 | Oligonucleotide primers used in this study.

Primer name	Sequence (5'–3')
MKM-GBK	GCCTCCCATGGTATAAATAGTGGCAAAGCGGGCCAAGGGCCCGGCCAAGAAGACCGCCAA GAAGACGACGGCCAAGTTTAAACGAGTCCAAGATCCGTCCGGTCACGCACGCGCTGCTCA AGGTGCGGCTGGACGGCAAATGTGCGAAAGCTACATATAAGGA
MKM BGC-DF:	TTTGCCTGGTGTTCATTC
MKMBGC-DR	AGTTCATGATGTTGACCGTG
METH-TRANS-FP	TACTAC CAT ATGCCCACCAGCATATTCTCCACCTC
METH-TRANS-RP	TACTAC CTCGAG TCAGCGCAGCCGTGC
MKM-DF	GTGAATGCCAGGAAGAGC
MKM-DR	CCAACCTGACCGGCTT
L2507	CCAGGATGTGATGAGCCG
23S_rRNA_F	CAAATTTTCGCAACACGATGATG
23S_rRNA_R	AAGGTTAAGCCTCACGG
T7_IR_AUG_F	TAATACGACTCACTATAGGGCTTAAGTATAAGGAGGAAAACATATG
yrbA_wt_F	TATAAGGAGGAAAACATATGATATACCCCTGCGGAGTGGGCGCGCG
yrbA_TTT_F	TATAAGGAGGAAAACATATGACCACGACCTGCGGAGTGGGCGCGCG
yrbA_SSS_F	TATAAGGAGGAAAACATATGAGCTCTAGCTGCGGAGTGGGCGCGCG
yrbA_wt_R	CCAAGTGTGAGGTGCGCCTAAAGCCGTTCAAGTTTGCATCGCGCGCCCACTC
yrbA_Ile_catch_R	CCAAGTGTGAGGTGCGCCTAAAGCCGTTCAAGTTTGCATCGCGCGCCCACTC
posT-NV1_R	GGTTATAATGAATTTTGCTTATTAACCTCGACATCGCATCAGGATTCAGCACGTGAATCCA ACTGTGCGAGGTCG
T7_gltX_F	TAATACGACTCACTATAGGGCATCTTTCTAAACGTAAG
gltX_NV1_R	GGTTATAATGAATTTTGCTTATTAACCCAGGAGTAAAGAGCTTAAGTACGCGCGCCGCC
NV1	GGTTATAATGAATTTTGCTTATTAA
MF_F1	ATTAATACGACTCACTATAGGGCAACCTAAACTTACACACGCCCCGGTAAGGAAATAAA AAT
MF_F2	GCCCCGGTAAGGAAATAAAAATGTTCAAAGCATTCAAAAACATCATACGTACTCGTACTC
MF_R	GGTTATAATGAATTTTGCTTATTAACCTTGCTGCGCTTAAAGAGTACGAGTACGTATGA TGT
T7_ermBL_F	TAATACGACTCACTATAGGGAGACTTAAGTATAAGGAGGAAAAAATATGTTGGTATTCCA AATGCGTAATGTAGATAA
ermBL_UGA_R	GGTTATAATGAATTTTGCTTATTAACGATAGAATTCTATCACTCAAATAGTAGATGTTTT ATCTACATTACGCATTT

Supplementary Table 9 | mRNA templates used in the study.

Template name	Sequence (5'–3')**	ORF translation
<i>yrbA</i> (wt)	AAUACGACUCACUAUAGGGCUUAAGUAUAAGGAGGA AAACAU AUGAUUAACCCUGCGGAGUGGGCGCGCA UCGCAAACUGAACGGCUUAG GCCGACCUCGACAGU UGGAUUCACGUGCUGAAUCCUGAUGCGAUGUCGA GU UAAUAAGCAAAAUUCAUUAUAACC	MIYPCGVGARSQ TERL*
<i>yrbA</i> _SSS	UAAUACGACUCACUAUAGGGCUUAAGUAUAAGGAGG AAAACAU AUGAGCUCUAGCUGCGGAGUGGGCGCGCG AUCGCAAUUGAACGGCUUAG GCCGACCUCGACAG UUGGAUUCACGUGCUGAAUCCUGAUGCGAUGUCGAG UUAUAAGCAAAAUUCAUUAUAACC	MSSSCGVGARSQ IERL*
<i>yrbA</i> _TTT	UAAUACGACUCACUAUAGGGCUUAAGUAUAAGGAGG AAAACAU AUGACCACGACCUGCGGAGUGGGCGCGCG AUCGCAAUUGAACGGCUUAG GCCGACCUCGACAG UUGGAUUCACGUGCUGAAUCCUGAUGCGAUGUCGAG UUAUAAGCAAAAUUCAUUAUAACC	MTTTCGVGARSQ IERL*
<i>gltX</i>	UAAUACGACUCACUAUAGGGCAUCUUUCUAAACGUA AGGCCAUUUC AUGAAAUCAAAACUCGCUUCGCGCC AAGCCCAACAGGCUAUCUGCACGUUGGCGGCGCGCG UACUUAAGCUCUUUACUCCUGG GUUAUAAGCAAAA UUCAUUAUAACC	MKIKTRFAPSPT GYLHVGGART*
<i>ermBL</i>	UAAUACGACUCACUAUAGGGAGACUUAAGUAUAAGG AGGAAAAAAU AUGUUGGUAUUCCAAUGCGUAAUGU AGAUAAAACAUCAUUAUUUGA GUGAUAGAAUUCUA UC GUUAUAAGCAAAAUUCAUUAUAACC	MLVFQMRNVDKT STI*
MFK	AUU AAUACGACUCACUAUAGGG CAACCUAAAACUUA CACACGCCCCGGUAAGGAAUAAAA AUGUUCAAAGC AUUCAAAAACAUCUACGUACUCGUACUCUUUAAGC GCAGGCAAG GUUAUAAGCAAAAUUCAUUAUAACC	MFKAFKNIIRTR TL*

** ORF in the template sequence is shown in red, T7 promotor is shown in blue, NV1 primer binding sequence – in green.

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