Supporting information

Inhibition of autotransporter biogenesis by small molecules

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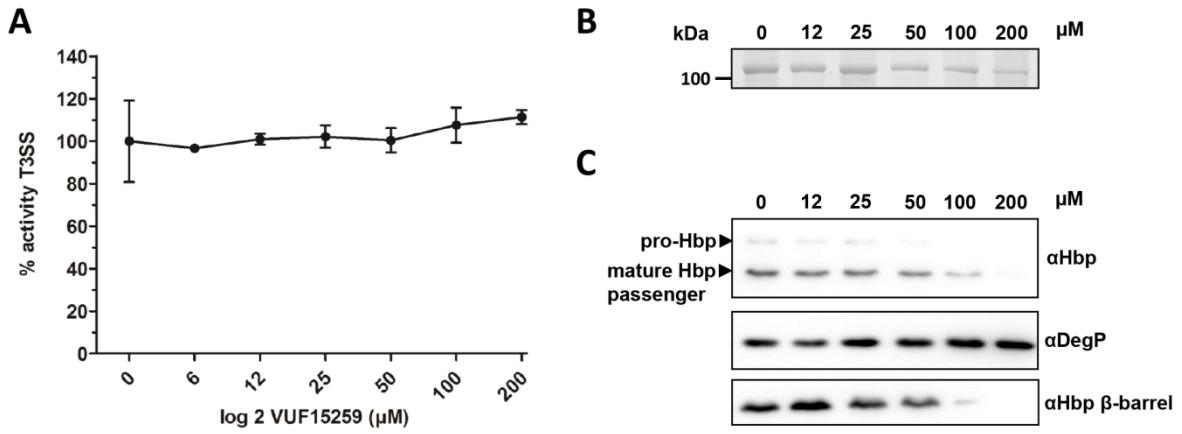


Fig S1. VUF15259 does affect T5SS in *Salmonella*, but not T3SS.

(A) *S. typhimurium* T3SS-dependent secretion was monitored by using a C-terminal NanoLuc (NL) luciferase (Hall *et al*., 2012) fusion of the T3SS effector protein SipA. *S. typhimurium* cells were grown in a 384-well plate in the presence of different concentrations of VUF15259. After 5 h, cells were removed by washing the wells, leaving the secreted SipA-NL bound to the walls of the high protein-binding plate to allow luminescence detection. The solvent DMSO served as positive control (the average set to 100% secretion activity) and a *S. typhimurium* Δ*invA* mutant, which is deficient in T3SS-dependent secretion, served as negative control (set to 0% secretion activity). Error bars represent the standard deviation of triplicate samples. (B) *S. typhimurium* T5SS-dependent secretion was monitored upon growth in a 96-well plate and incubation with the indicated VUF15259 concentrations. Hbp was expressed from the pEH3 plasmid by adding 40 µM IPTG. After 3 h cells were separated from spent medium by centrifugation. The spent medium was TCA precipitated and analyzed by SDS-PAGE and Coomassie staining. Secretion of Hbp appeared impaired by VUF15259 as indicated by a dose-dependent decrease in the amount of secreted Hbp passenger. (C) Cells were analyzed by SDS-PAGE and western blotting. In agreement with the inhibitory effect of VUF15259 on Hbp secretion we observed a dose-dependent decrease in mature Hbp passenger and corresponding β-barrel domain. Accumulation of pro-Hbp was not detected, most likely due to degradation by the protease DegP that appeared upregulated.

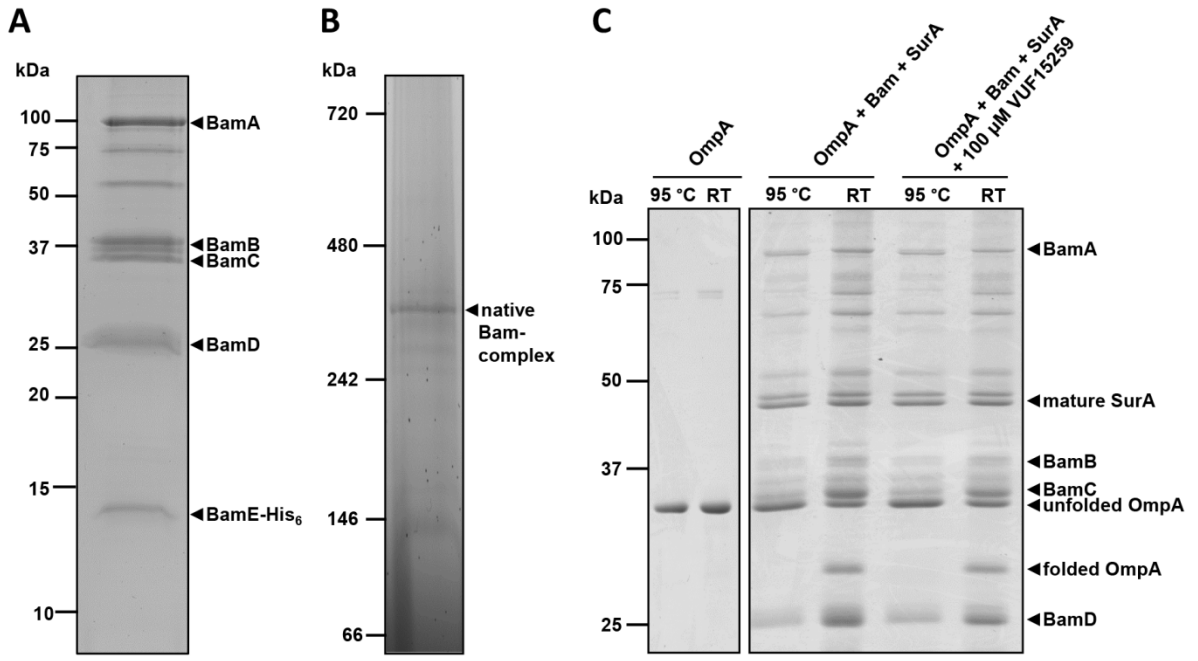


Fig S2. VUF15259 does not affect OmpA insertion and folding into proteoliposomes.

E. coli BL21 (DE3) strain harboring expression plasmids for all Bam subunits were grown, induced with 0.4 mM IPTG at mid log phase and growth was continued for 2 h. Cells were separated from medium by centrifugation and an enriched outer membrane fraction was isolated. The Bam-complex was purified from this fraction using Ni-NTA resin making use of a His_6-tag fused to BamE. Elution fractions were pooled and analyzed by (A) 15% SDS-PAGE and Coomassie staining, and (B) 3-12% Blue-native PAGE. Of note, BamA degradation products were observed between 50 and 75 kDa. The native Bam-complex was detected between 480 and 242 kDa as reported (Roman-Hernandez, Peterson, & Bernstein, 2014). (C) The Bam-complex was incorporated into liposomes and the resulting proteoliposomes were used to monitor insertion and folding of OmpA. OmpA was cloned and expressed without its ss from the pET22b plasmid resulting in inclusion bodies that were isolated and dissolved in 8 M urea to completely denature OmpA. SurA was expressed from the pET22b plasmid and purified using a C-terminal His_6-tag. Urea-denatured OmpA was pre-incubated with SurA for 20 min following a 2 h incubation at 30 °C in the presence of empty liposomes or proteoliposomes that were pre-incubated with VUF15259 or DMSO. The samples were analyzed by semi-native SDS-PAGE. To examine folding of OmpA the heat-modifiability of OmpA was tested by incubating the samples either at room temperature (RT) or at 95 °C for 10 min. BamE is not visible on this gel, due to the low molecular mass of the protein.

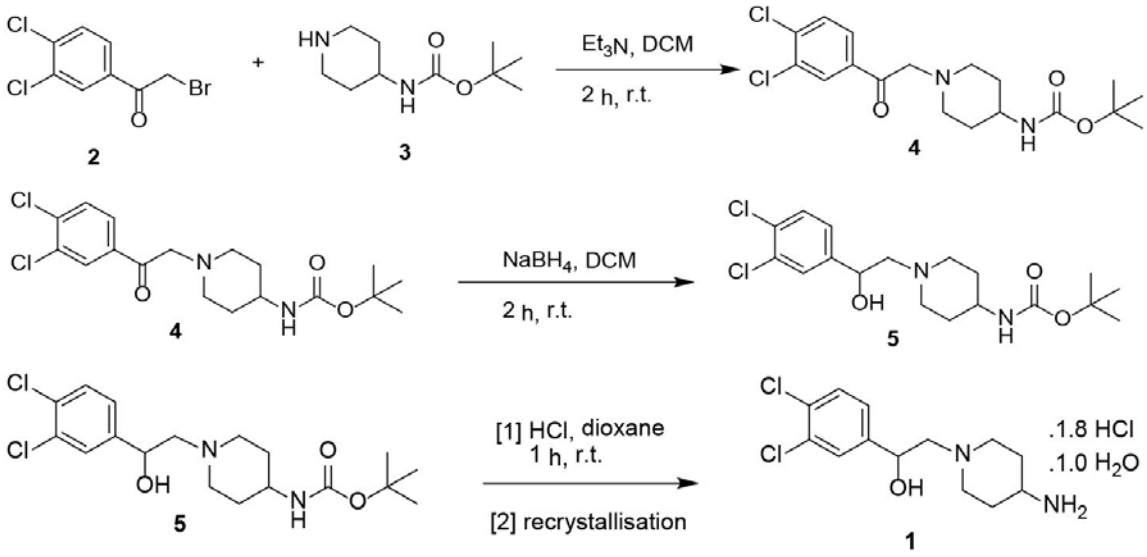


Fig S3. Synthetic approach to key compound VUF15259.
Detailed procedures are described in the materials and methods section.

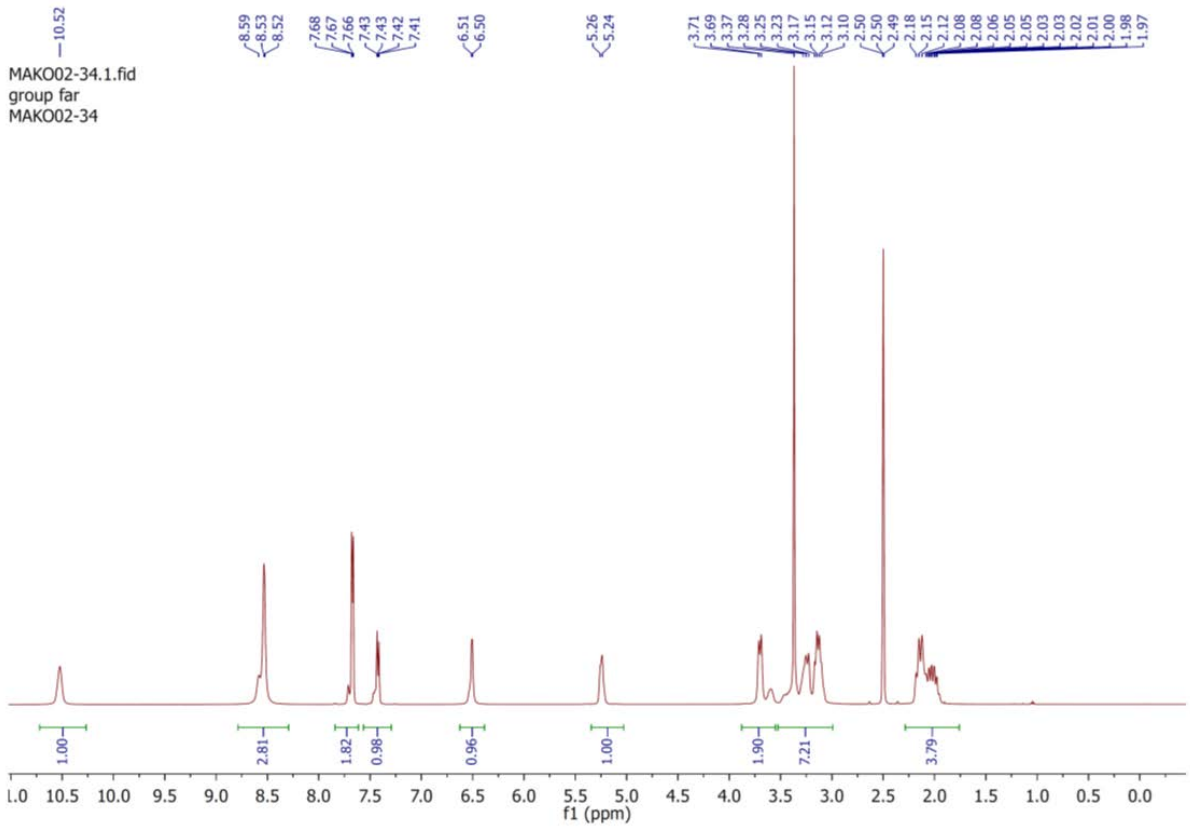


Fig S4. ^{1}H NMR spectrum of 1 (VUF15259) in DMSO-d_6.

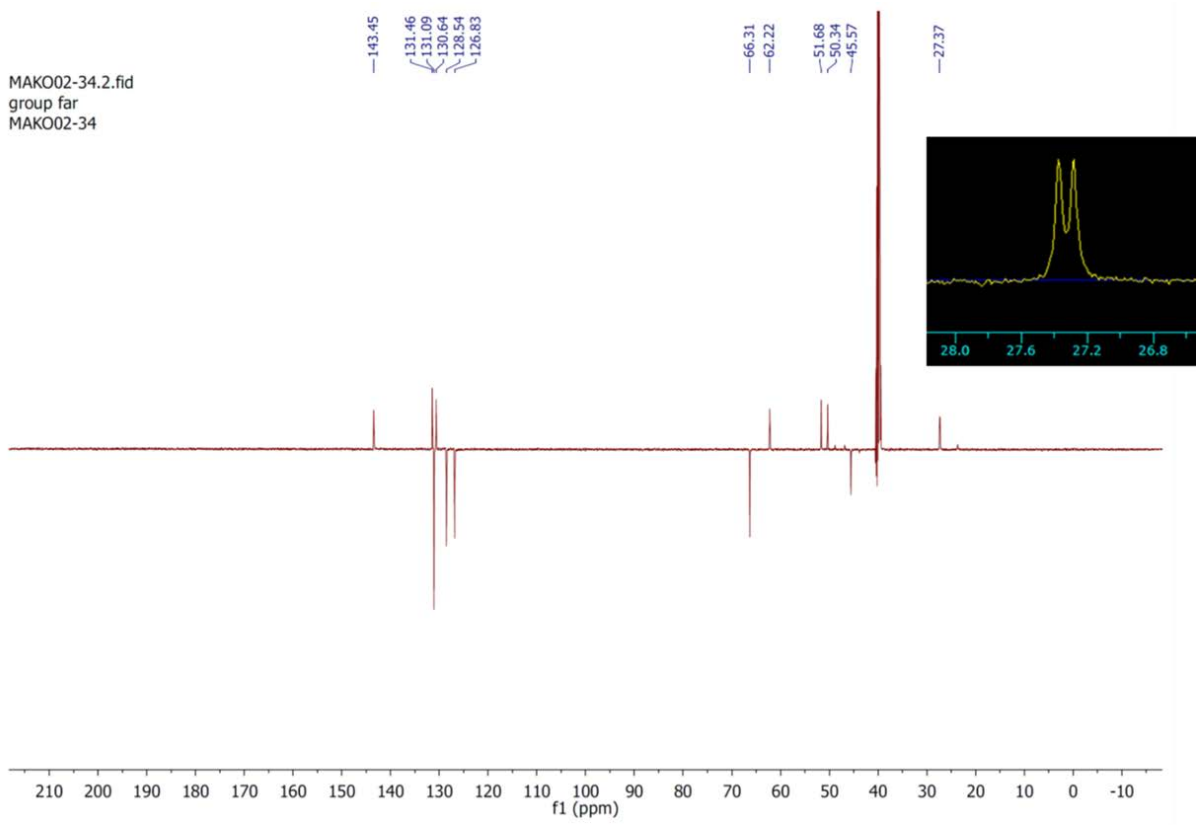


Fig S5. ^{13}C NMR spectrum of 1 (VUF15259) in DMSO-d_6.
The inset shows a blow-up of the 28.0-26.8 region to highlight two almost overlapping signals.

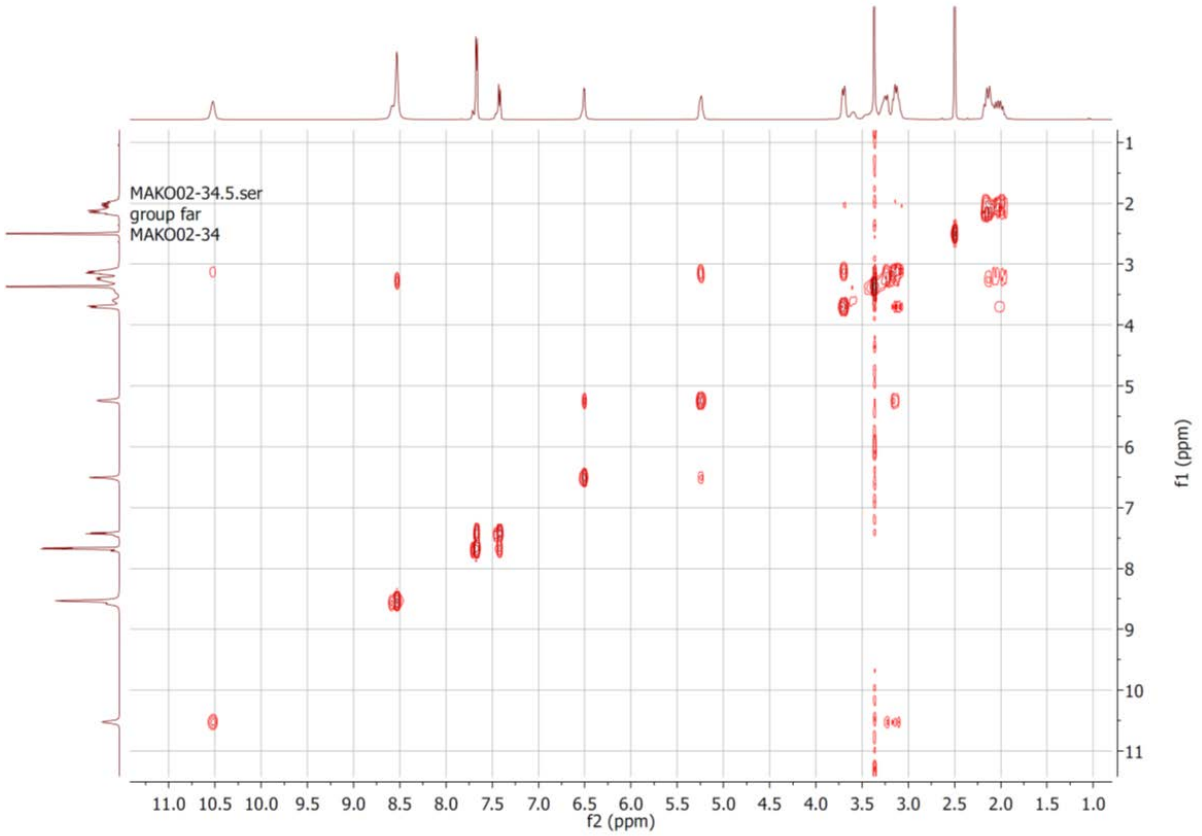


Fig S6. COSY spectrum of 1 (VUF15259) in DMSO-d6.

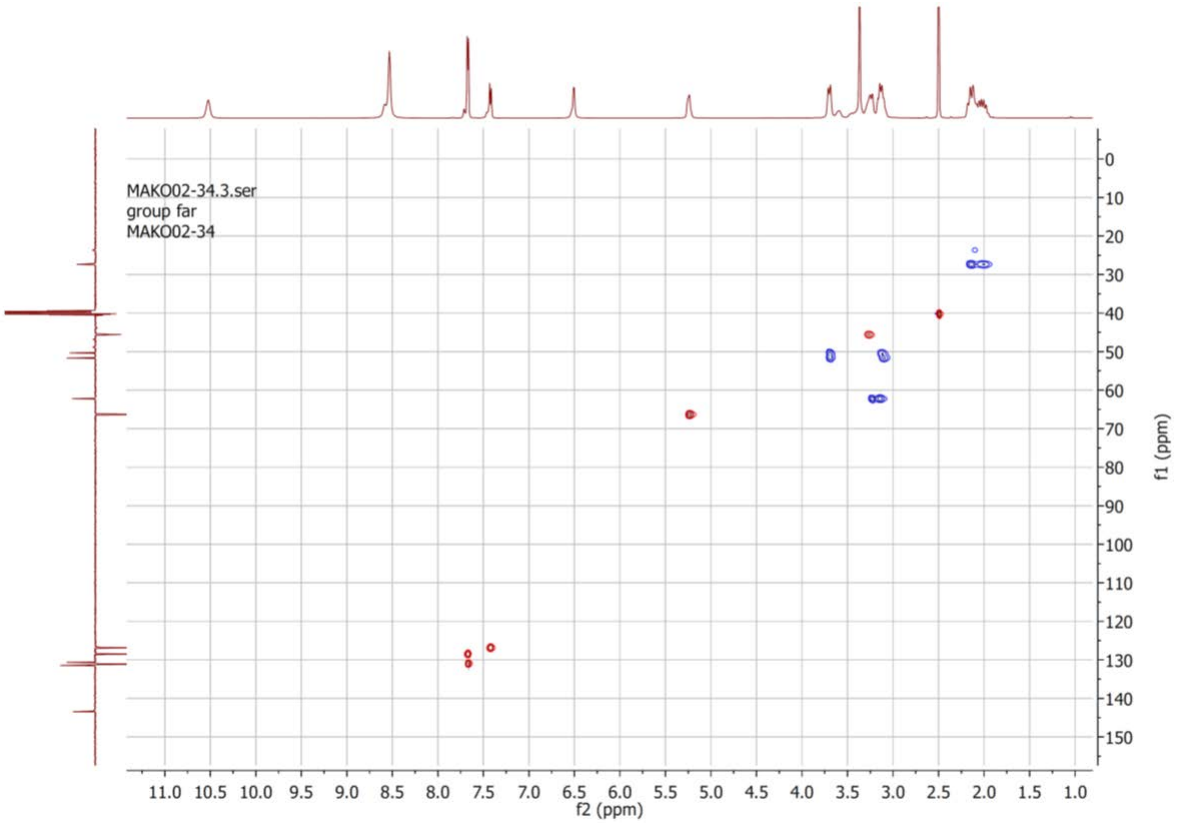
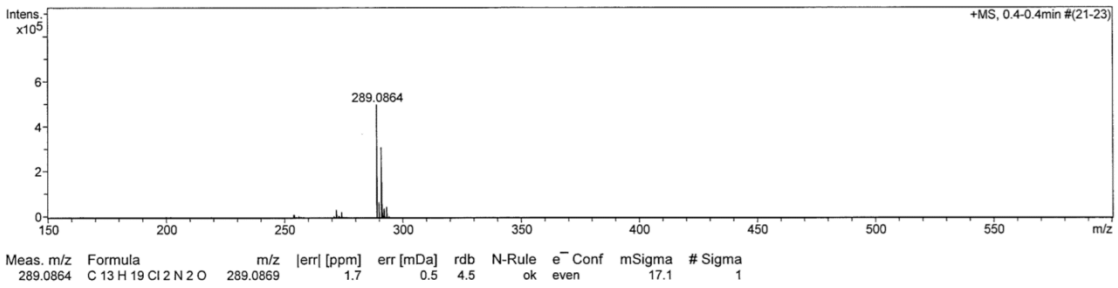


Fig S7. HSQC spectrum of 1 (VUF15259) in DMSO-d6.



Meas. m/z	Formula	m/z	|err| [ppm]	err [mDa]	rdb	N-Rule	e¯ Conf	mSigma	# Sigma
289.0864	C 13 H 19 Cl 2 N 2 O	289.0869	1.7	0.5	4.5	ok	even	17.1	1

Fig S8. HRMS spectrum of 1 (VUF15259).

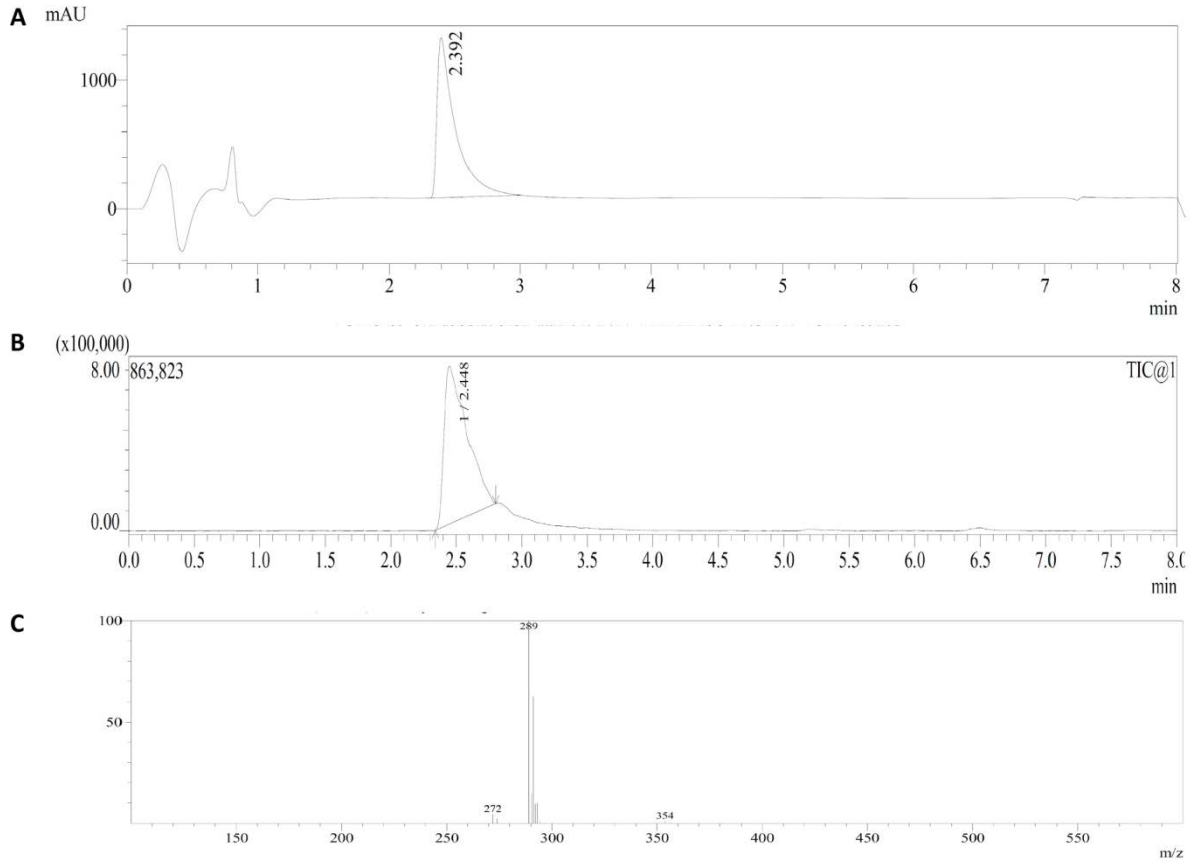


Fig S9. LC-MS analysis of 1 (VUF15259).

(A) UV detection at 230 nm. A relatively high solvent front due to the very low epsilon value of compound 1 is visible and cannot be avoided. (B) TIC MS detection. (C) ESI-MS spectrum of main eluted peak.

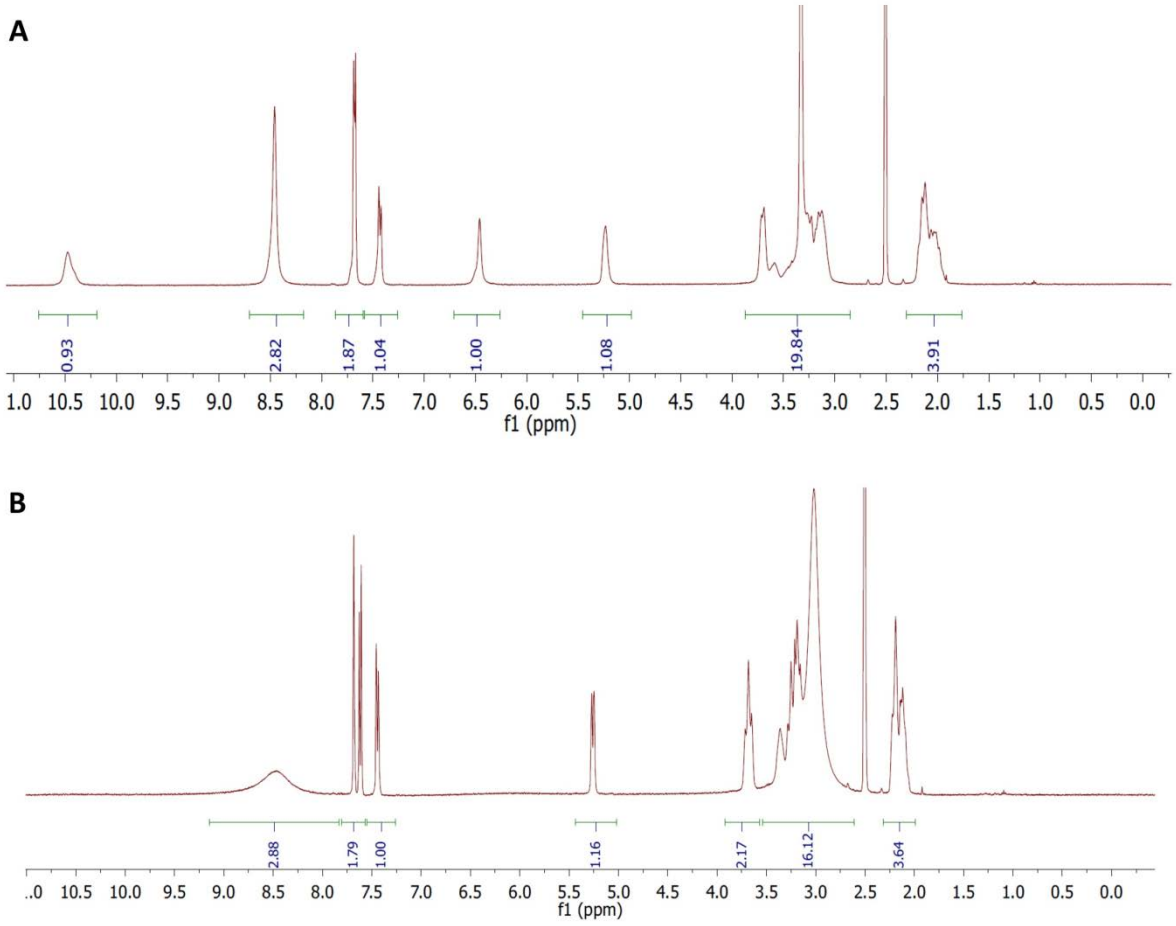


Fig S10. Effect of temperature on the ^{1}H-NMR spectrum of 1 (VUF15259) in DMSO-d6.

(A) Room temperature. (B) 100 °C. Note: these spectra were recorded on a different batch of the compound than the batch shown in all the other spectral figures.

Table S1. Strains used in this study.

***E. coli* strain**	**Description**	**Reference**
MC1061	Cloning and expression strain	Thermo Scientific
MC1061 *degP::S210A*	Mutation rendering DegP defective in its protease function	(Spiess, Beil, & Ehrmann, 1999)
TOP10F'	Cloning and expression strain	Thermo Scientific
MC4100	Cloning and expression strain	(Taschner, Huls, Pas, & Woldringh, 1988)
MC4100 Δ*surA*	Deletion of the chaperone *surA*	(Baba *et al*., 2006)
MC4100 Δ*bamB*	Deletion of the protein *bamB*	(Baba *et al*., 2006)
MC4100 Δ*surA*/Δ*bamB*	Deletion of the chaperone *surA* and protein *bamB*	(Baba *et al*., 2006)
KS272	Parent strain KS474	(Strauch & Beckwith, 1988)
KS474 Δ*degP*	Deletion of the protease *degP*	(Strauch & Beckwith, 1988)
BL21 (DE3)	Cloning and expression strain	Novagen

Table S2. Plasmids used in this study.

Plasmid name	Description	Reference
pEH3	Expression vector; *lac*UV5 promoter	(Hashemzadeh-Bonehi *et al*., 1998)
pEH3-Hbp	P_{lac}-*hbp*	(Jong *et al*., 2007)
pEH3-Hbp110C/348C	P_{lac}-*hbp110C/348C*	(Jong *et al*., 2007)
pEH3-ssTorA-Hbp	P_{lac}-*hbp* with *torA* signal sequence	(Jong *et al*., 2017)
pEH3-PhoE	P_{lac}-*phoE*	(Jong *et al*., 2010)
pEH3-Antigen-43	P_{lac}-*antigen-43*	This study
pEH3-RpoE	P_{lac}-*rpoE*	This study
pUA66	Cloning vector containing *gfpmut2*	(Zaslaver *et al*., 2006)
pUA66-RpoE-NeonGreen	P_{rpoE}-*neongreen*	This study
pUA66-GroES-NeonGreen	P_{groES}-*neongreen*	This study
pET-BamCDE-His_6	Expression vector for *bamC*, *bamD* and *bamE* under P_{T7}	This study
pCDF-BamAB	Expression vector for *bamA and bamB* under P_{T7}	This study
pET22b-SurA	P_{T7}-*surA*-His_6 (21-408)	This study
pET22b-OmpA	P_{T7}-*ompA* (22-346)	This study
pLEMO	Expression of T7 lysozyme under P_{Rha}	(Schlegel *et al*., 2013)

Table S3. Primers used in this study.

Primer[†]	**Sequence (5' → 3')**[‡]
mNeonGreen fw optimized	gacttctagatttaagaaggagatatacatatggtaagtaaaggtgaag
mNeonGreen rv	tcagctctagagcttgcatgcctgcaggtctggacatttagaattccttgtacagctc
P*rpoE* fw	tcgactcgagcagcatgacaaacaaaaac
P*rpoE* rv	tgcaggatccttagctgcaatttgagcaagc
P*groES* fw	tcgactcgagctgatcagaatttttttctttttc
P*groES* rv	tgcaggatcctacgtggtttcccggctgg
rpoE fw	actgggatccatgagcgagcagttaacgg
rpoE rv	atcgggtacctcaacgcctgataagcgg
antigen-43 fw	taactttctagattacaaaacttaggagggtttttaccatgaaacgacatctgaatacc
antigen-43 rv	gactgaattcggatcctcagaaggtcacattcagtgtg
bamA FW	atatcatatggcgatgaaaaagttgc
bamA RV	atatagatctttaccaggttttaccgatgttaaac
bamB FW	atatccatgggattgcgtaaattactgctgc
bamB RV	atataagcttttaacgtgtaatagagtacacggttcc
bamC FW	atatccatggcttactctgttcaaaagtc
bamC RV	atataagctttacttgctaaacgcagc
bamD FW	atatcatatgacgcgcatgaaatatctg
bamD RV	atatagatctttatgtattgctgctgtttgc
bamE FW	atatagatcttataagaaggagatactagtatgcgctgtaaaacgctg
bamE-His$_6$ RV	atatctcgagttagtgatgatgatgatgatgatgatggttaccactcagcgcagg
surA FW	tcgacatatgccccaggtagtcgataaagtcg
surA RV	atatctcgaggttgctcaggattttaacgtagg
ompA FW	tcgacatatggactacaaagacgatgacgacaaggctccgaaagataacacctg
ompA RV	tcgactcgagttaagcctgcggctgag

[†]FW: forward; RV: reverse

[‡]Restriction sites used for cloning are underlined and C/G to A/T substitution are depicted in red

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