Supplemental Material

Table S1. Bacterial strains and plasmids used in this study.

Strain or plasmid	Description	Reference
Strains		
E. coli		
XL1 Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F' *proAB lacIqZΔM15*::Tn10 (Tet^r)]	Stratagene
W3110	Wild-type, F^-I^- rph-1 INV(*rrnD, rrnE*)1 *rph-1*	*E. coli* Genetic Stock Center (Yale)
W3110Δ*eptA* (CH030)	W3110, Δ*eptA*	(Herrera *et al.*, 2010)
BN2	K-12 BW25113, Δ*pagP*, Δ*lpxT*, Δ*eptA*, Δ*lpxM*	(Needham *et al.*, 2013)
SM10	*thi-1 thr leu tonA lacY supE recA*:: RP4-2-Tc::Mu (Kan^r)	(de Lorenzo and Timmis, 1994)
P. aeruginosa		
PA14	Wild-type, UCBPP-PA14	(Liberati *et al.*, 2006)
PA14Δ*eptA*$_{Pa}$	PA14, Δ*eptA*$_{Pa}$	This study
PA14Δ*colR*	PA14, Δ*colR*	This study
PA14Δ*colS*	PA14, Δ*colS*	This study
Plasmids		
pACYC184	Low copy-number cloning vector, tet^R, cam^R	Novagen
pPA14_58610	pACYC184 containing PA14_58610 coding sequence and RBS	This study
pPA14_21210	pACYC184 containing PA14_21210 coding sequence and RBS	This study
pPA14_39020 (pAC*eptA*$_{Pa}$)	pACYC184 containing PA14_39020 (*eptA*$_{Pa}$) coding sequence and RBS	This study
pWSK29	Low copy vector, T7 and T3 RNA polymerase promoters, ampicillin resistance	(Wang and Kushner, 1991)
p*lpxE*$_{Fn}$	pWSK29 containing *F. novicida lpxE*	(Wang *et al.*, 2004)
p*lpxF*$_{Fn}$	pWSK29 containing *F. novicida lpxF*	(Wang *et al.*, 2006)
pEX1.8	Medium copy-number cloning vector, pEX1 carrying *ori* (*P. aeruginosa*) as 1.8-kb (Pearson *et al.,* 1997)(8)(7)(6)(5)*Pst*I fragment from pRO1614 in *Sty*I site	(Pearson *et al.*, 1997)
p*eptA*$_{Pa}$	pEX1.8 containing *eptA*$_{Pa}$ coding sequence and RBS	This study
p*eptA*$_{nprom}$	pEX1.8 containing *eptA*$_{Pa}$ coding sequence and native promoter	This study

Strain or plasmid	Description	Reference
p*pmrA*	pEX1.8 containing *pmrA* coding sequence and RBS	This study
p*phoP*	pEX1.8 containing *phoP* coding sequence and RBS	This study
p*colR*	pEX1.8 containing *colR* coding sequence and RBS	This study
p$colR_{nprom}$	pEX1.8 containing *colR* coding sequence and native *colRS* promoter	This study
p$colS_{nprom}$	pEX1.8 containing *colS* coding sequence and native *colRS* promoter	This study
pEX18Gm	Suicide (gene replacement) vector, *oriT*+ *sacB*+, pUC18 MCS, gentamycin resistance	(Hoang *et al.*, 1998)
pEX18Gm:*eptA*del	pEX18Gm containing assembly PCR product of 1Kb upstream and downstream flanking regions of *eptA* coding sequence	This study
pEX18Gm:*colR*del	pEX18Gm containing assembly PCR product of 1Kb upstream and downstream flanking regions of *colR* coding sequence	This study
pEX18Gm:*colS*del	pEX18Gm containing assembly PCR product of 1Kb upstream and downstream flanking regions of *colS* coding sequence	This study

Table S2. Oligonucleotides used in this study.

Primer	Sequence (5′-3′)	Application
5′ PA14_58610	GCGATATCCCATCGAACGAGGCTATCGTGTC	Used to clone PA14_58610 and native RBS into pACYC184
3′ PA14_58610	CGCGGATCCGCGTCAGCCTTCGTTCGGCTG	
5′ PA14_21210	CGAGCGATATCTCAGGAGTTGCTTCAATGGGT	Used to clone PA14_21210 and native RBS into pACYC184
3′ PA14_21210	CGCGGATCCTCACTGGGCTGCCCTGGG	
5′ PA14_39020	CGAGCGATATCCAGTGAAGATCCGTGCCCATG	Used to clone PA14_39020 ($eptA_{Pa}$) and native RBS into pACYC184
3′ PA14_39020	CGATGTCGACTCAGGAAGCCGGC	

Primer	**Sequence (5′-3′)**	**Application**
	GGCTCCT	
5′ PA*eptA*	CGAGCGAATTCCAGTGAAGATCCG TGCCCATG	Used to clone $eptA_{Pa}$ into pEX1.8
3′ PA*eptA*	CGATAAGCTTTCAGGAAGCCGGCG GCTCCT	
5′ *eptA*outdel	GAGAGCTCGAAGCGTCCGACGGT GTCCAG	Used to clone upstream $eptA_{Pa}$ flanking region and for assembly PCR to generate pEX18Gm:*eptA*del
3′ *eptA*outdel	GAGGATCCGTCCCGATGAACCACC CGCA	Used to clone downstream $eptA_{Pa}$ flanking region and for assembly PCR to generate pEX18Gm:*eptA*del
5′ *eptA*indel	GATCCGTGCCCATGTCGAAAGCCG AGGAAGCGTCTGGCCAGGAG	Used to clone downstream $eptA_{Pa}$ flanking region
3′ *eptA*indel	CTCCTGGCCAGACGCTTCCTCGGC TTTCGACATGGGCACGGATC	Used to clone upstream $eptA_{Pa}$ flanking region
5′ PA$eptA_{nprom}$	ACGCGTCGACGAAATTCACAGAAG GGTTTCCCG	Used to clone $eptA_{Pa}$ and native promoter (nprom) into pEX1.8
3′ PA$eptA_{nprom}$	CAAGACGTCGACTCGCCGGGTCG TATCAGGAA	
5′ PA*pmrA*	CGGAATTCATGAGAATACTGCTGG CCGAGGACGACCT	Used to clone *pmrA* into pEX1.8
3′ PA*pmrA*	CCCAAGCTTTCAGGGCGCCGGCT GGTC	
5′ PA*phoP*	CGGAATTCATGAAACTGCTGGTAG TGGAAGACGAGG	Used to clone *phoP* into pEX1.8
3′ PA*phoP*	CCCAAGCTTTCACCGGCAGCGCTC GGTG	
5′ PA*colR*	CGGAATTCATGCGAATACTGGTGG TCGAAG	Used to clone *colR* into pEX1.8
3′ PA*colR*	CCCAAGCTTTTATACTCCATTCGGC TCCTCC	
5′ *colR*outdel	GTTCTACATGAAGGTGCCCATCGA GTTCGG	Used to clone upstream *colR* flanking region and for assembly PCR to generate pEX18Gm:*colR*del
3′ *colR*outdel	CGCAGCAGTGGCGCGTTGAACTG	Used to clone downstream *colR* flanking region and for assembly PCR to generate pEX18Gm:*colR*del
5′ *colR*indel	GGACATGCGAATACTGGTGGAGCC	Used to clone downstream

Primer	Sequence (5′-3′)	Application
	GAATGGAGTATAAGC	*colR* flanking region
3′ *colR*indel	GCTTATACTCCATTCGGCTCCACC AGTATTCGCATGTCC	Used to clone upstream *colR* flanking region
5′ *colS*outdel	CGGAATTCCGAGGAACAGCCAGG GGTTGAATGGAC	Used to clone upstream *colS* flanking region and for assembly PCR to generate pEX18Gm:*colS*del
3′ *colS*outdel	CGGGATCCCACGCGAATACCAAGC GACTCGAAGGC	Used to clone downstream *colS* flanking region and for assembly PCR to generate pEX18Gm:*colS*del
5′ *colS*indel	GAGGAGCCGAATGGAGTATAAGCT CGATGTTGCTTGACGA	Used to clone downstream *colS* flanking region
3′ *colS*indel	TCGTCAAGCAACATCGAGCTTATA CTCCATTCGGCTCCTC	Used to clone upstream *colS* flanking region
5′ PA*colR*$_{nprom}$	ACGCGTCGACCGATCACCAGTTGC TTGAC	Used to clone *colR* and native promoter (nprom) into
3′ PA*colR*$_{nprom}$	GAGGAGCCGAATGGAGTATAAAAG CTTGC	pEX1.8
5′ PA*colS*$_{nprom}$	CGCGGATCCCGATCACCAGTTGCT TGAC	Used to clone *colRS* native promoter (nprom) into
3′ PA*colS*$_{nprom}$	GCGAATTCGTCCCACTCCTTCGCA GGA	pEX1.8
5′ PA*colS*	GCGAATTCATGGAGTATAAGCAGA GCCTCGC	Used to clone *colS* into pEX1.8
3′ PA*colS*	GGAAGCTTTCAAGCAACATCGAGT AAAACTTCGAACC	
5′ q*clpX*	AAGAAGGTTCTGGCGGTAGC	qPCR analysis of *clpX*
3′ q*clpX*	ATGTTCTCGACATCCTCGCC	transcription
5′ q*eptA*	TGCCCTGCATGTTCTCCAAC	qPCR analysis of *eptA*
3′ q*eptA*	GATCCTTGCTCTCGCTCAGG	transcription
5′ q*arnT*	GGCTATGCCAACCTCGACCC	qPCR analysis of *arnT*
3′ q*arnT*	GCGAGGAAGCCCTTGGTCAG	transcription

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Supplemental Figures:

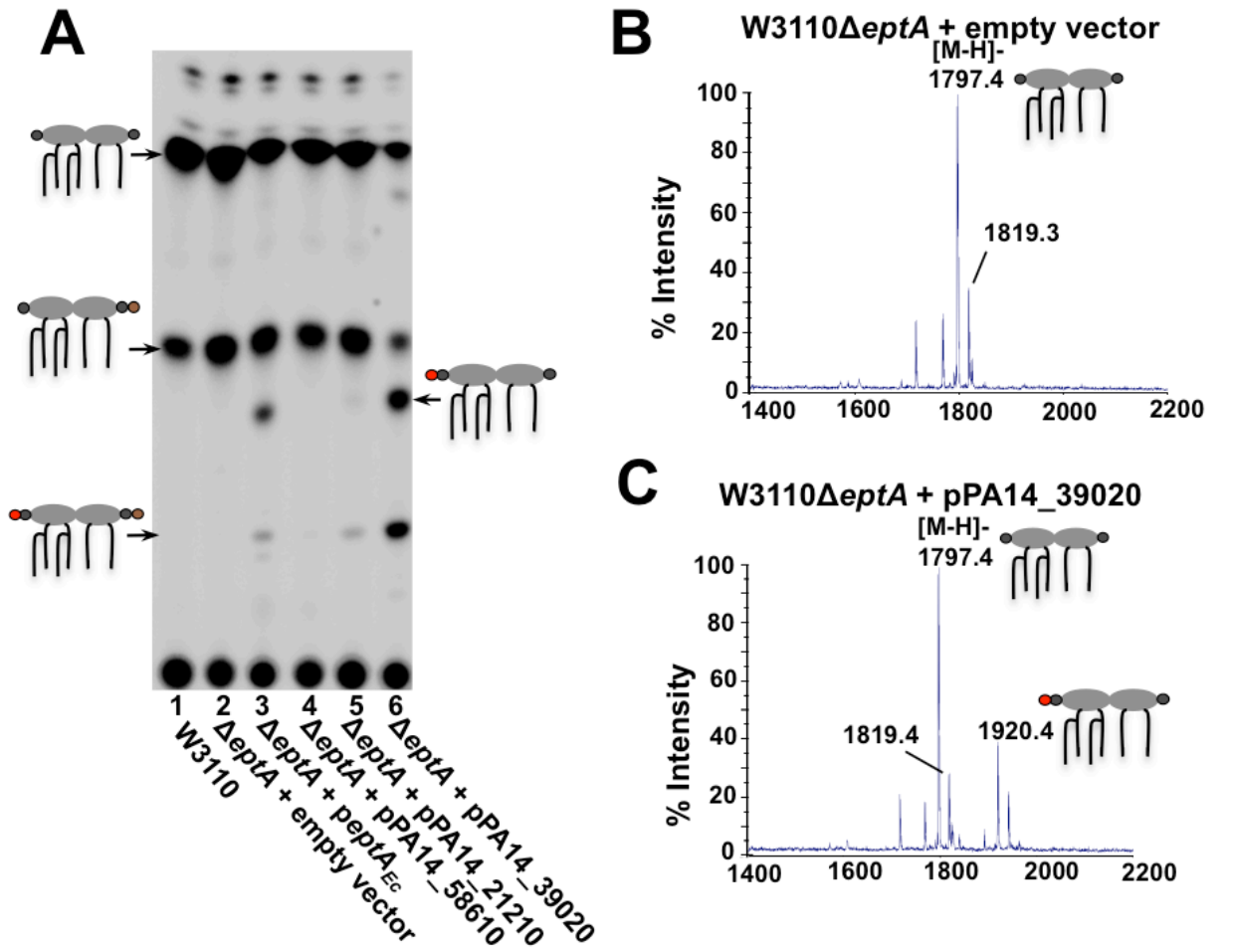

Fig S1. Identification of a functional *P. aeruginosa* pEtN transferase ($eptA_{Pa}$) in *E. coli*. A) *P. aeruginosa eptA* orthologs were heterologously expressed in K-12 *E. coli* strain W3110Δ*eptA*. PA14_39020 and $eptA_{Ec}$ were able to modify lipid A. B) MALDI-TOF MS analysis of lipid A isolated from Δ*eptA* + empty vector shows no pEtN addition to lipid A, while (C) expression of PA14_39020 reveals pEtN modification.

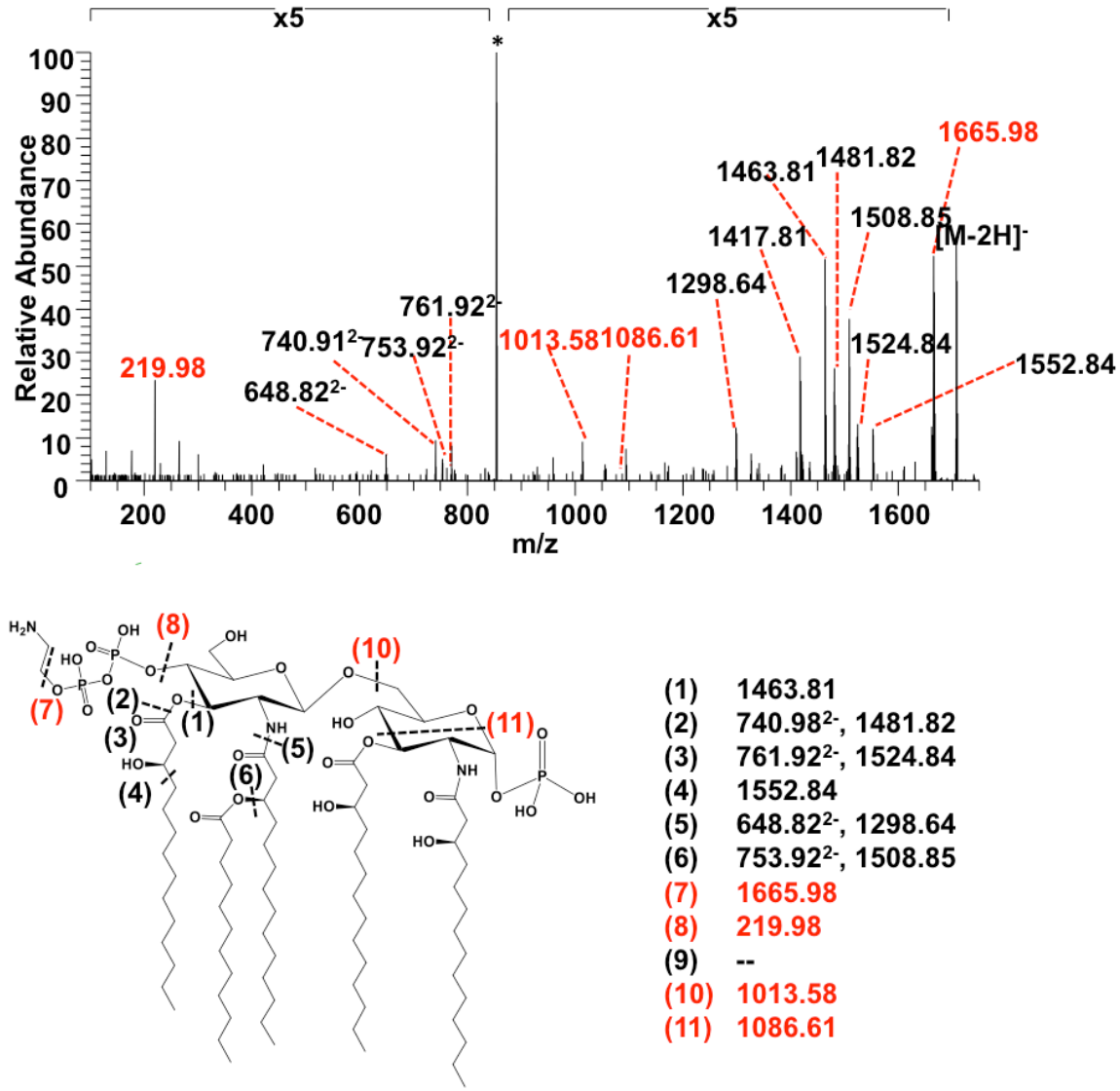

Fig S2. UVPD mass spectrum and fragmentation map of doubly deprotonated lipid A prepared from BN2 + p*eptA*$_{Pa}$ [M_r = 1710.03]. Key fragments and cleavage sites that support the identification and location of the 4′ modification are shown in red font. The precursor ion is marked with an asterisk.

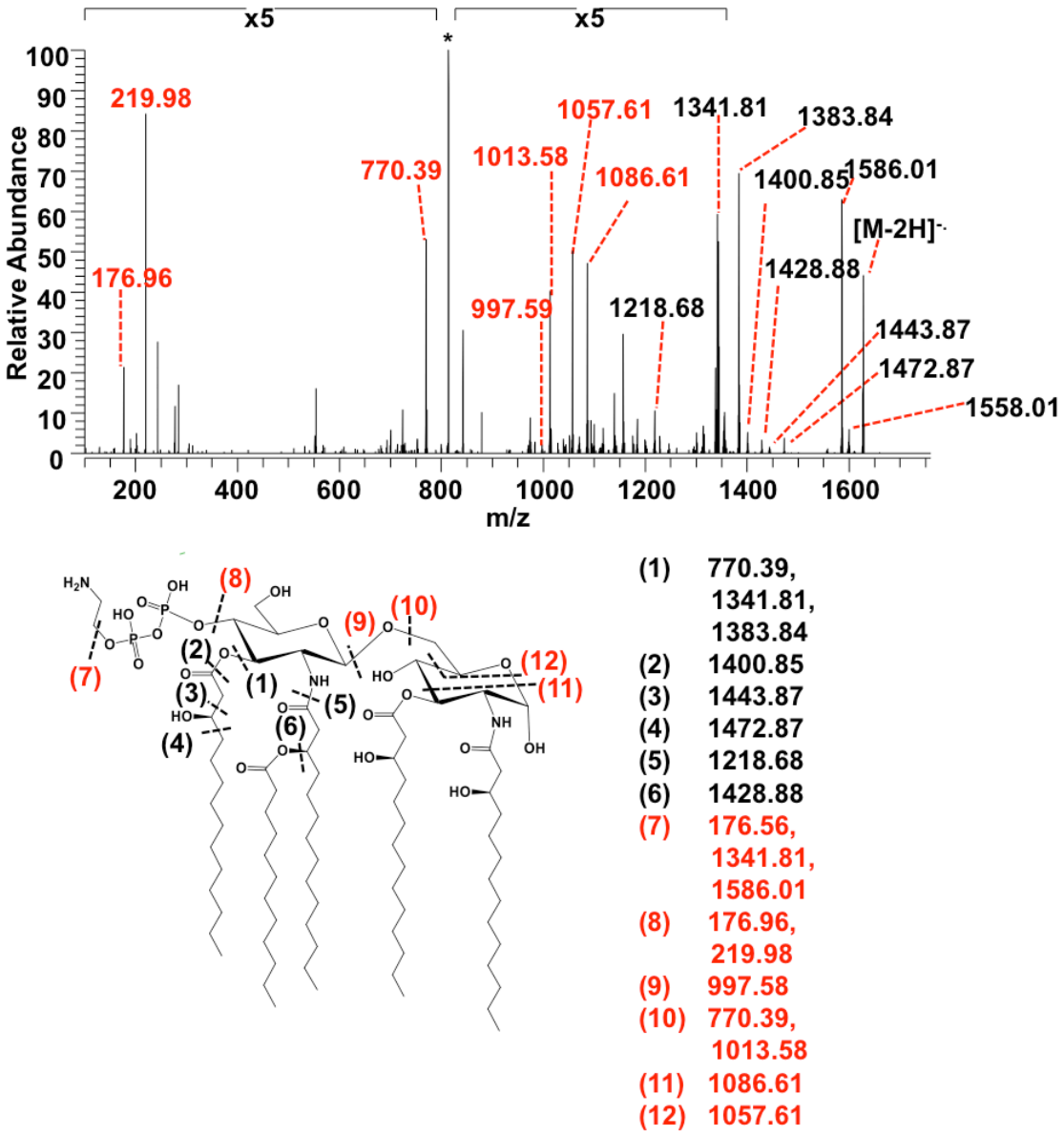

Fig S3. UVPD mass spectrum and fragmentation map of doubly deprotonated lipid A prepared from BN2 + p*eptA*$_{Pa}$ + p*lpxE* [M_r = 1630.06]. Key fragments and cleavage sites that support the identification and location of the 4′ modification are shown in red font. The precursor ion is marked with an asterisk.

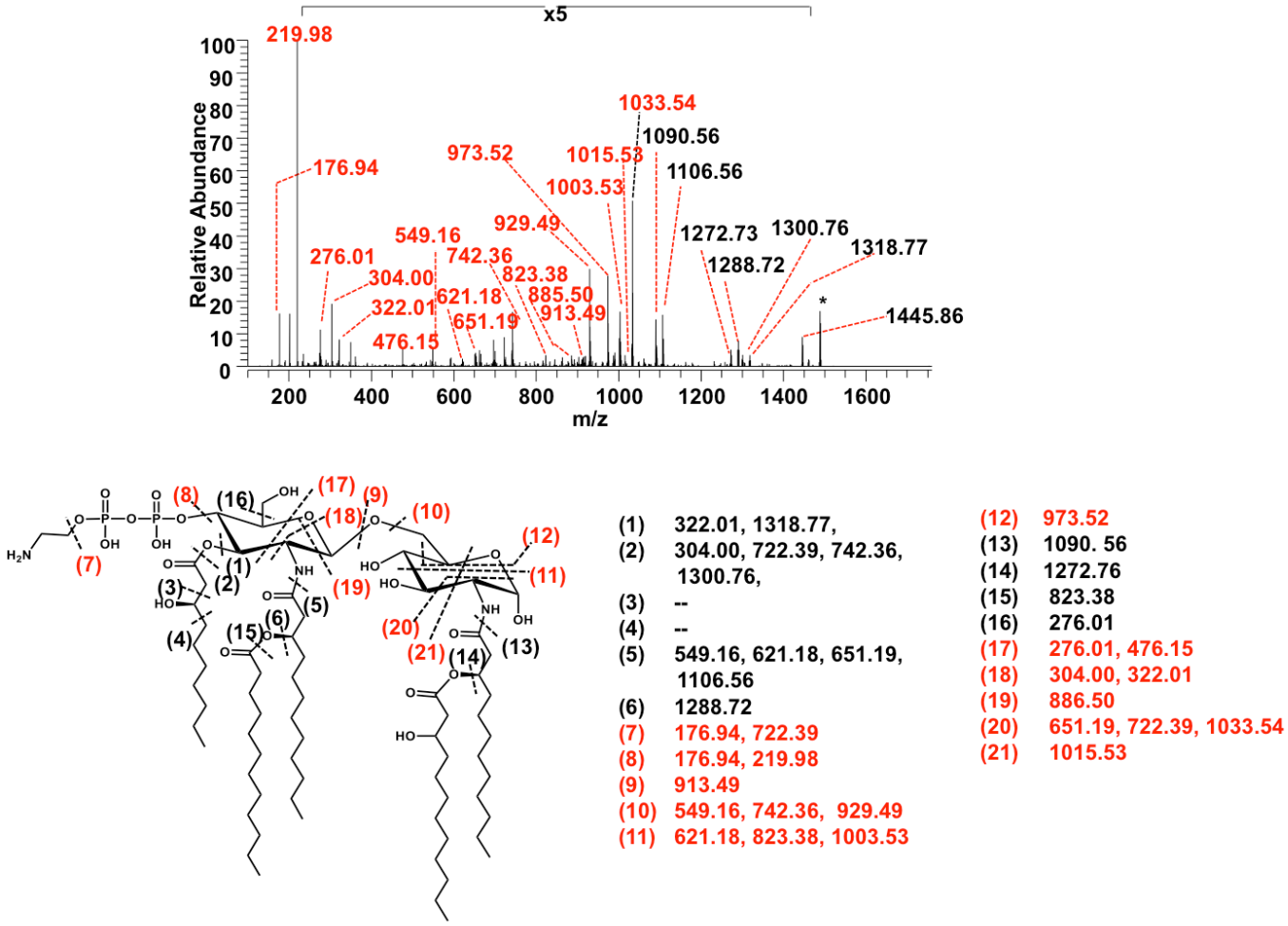

Fig S4. UVPD mass spectrum and fragmentation map of singly deprotonated lipid A prepared from PA14 + peptAPa [M_r = 1489.91]. Key fragments and cleavage sites that support the identification and location of the 4′ modification are shown in red font. The precursor ion is marked with an asterisk.

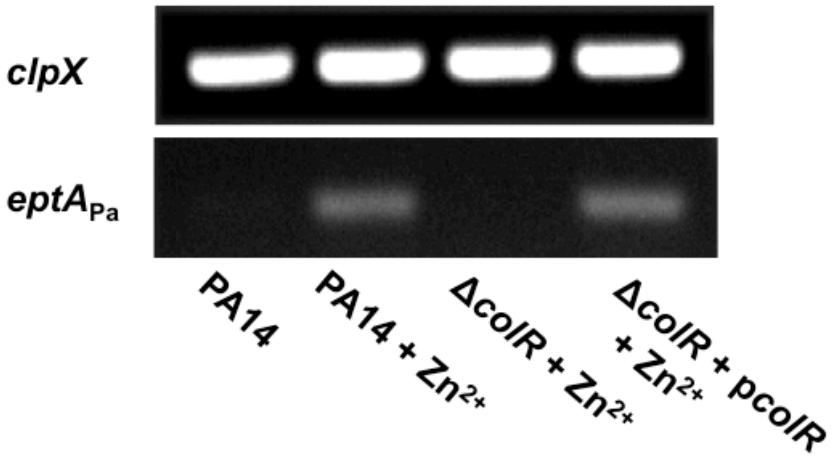

Fig S5. Excess zinc induces transcription of *eptA*$_{Pa}$. Semi-quantitative RT-PCR shows no detectable *eptA*$_{Pa}$ transcription in PA14 cells grown in LB alone, while addition of 1mM $ZnSO_4$ results in detectable transcription. No transcription of *eptA*$_{Pa}$ is visualized in PA14Δ*colR* grown in LB with 1mM $ZnSO_4$. When this mutant is complemented, *eptA*$_{Pa}$ transcription in response to 1mM $ZnSO_4$ can once again be seen. The stably expressed housekeeping gene *clpX* was used as a control.

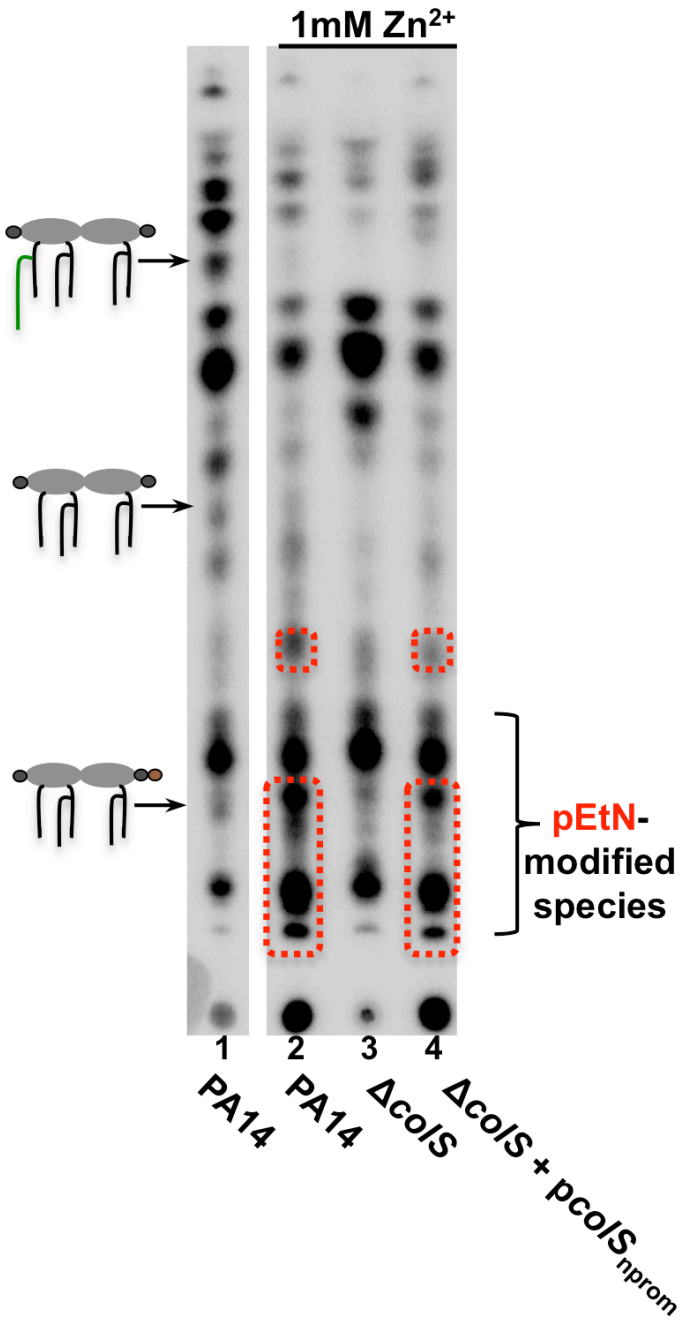

Fig S6. Deletion of *colS* results in loss of Zn^{2+}-induced pEtN modification of *P. aeruginosa* lipid A. Lipid A was isolated from ^{32}P-labeled cells grown in LB broth and separated by TLC. Lipid A species modified with pEtN were observed in PA14 supplemented with 1mM $ZnSO_4$ (lane 2). Deletion of *colS* resulted in no pEtN modification of lipid A in response to Zn^{2+} (lane 3). Modification is restored in the mutant complemented with *colS* (lane 4).