Supplemental Data

Supplementary Experimental Procedures

Escherichia coli strains.

TB1 - F^- *ara, Δ(lac-pro*AB*), [Φ80dlac, Δ(lacZ)M15] rps*L*(Str^R), thi, hsd*R (1), obtained from Dr A.J.Lloyd. **Top10 F** - mcrA Δ(mrr-hsdRMS-mcrBC) ϕ80lacZΔM15 ΔlacX74 deoR recA1 araD139 Δ (ara-leu) 7697 galU galK rpsL (Str^R) endA1 nupG (2), obtained from Invitrogen. **BL21 Star (λDE3)** - F^- *ompT hsdS*$_B$ (r_B^- m_B^-) *gal dcm rne*131 (λDE3) (3), obtained from Dr D. I. Roper. **C41 (DE3)** - derivative of *E. coli* BL21(λDE3): F^- *ompT hsdS*$_B$ (r_B^- m_B^-) *gal dcm* (DE3) (4). **B834** - F^- *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm met* (DE3) (5).

Escherichia coli plasmids.

pBADM41 - a vector carrying ampicillin resistance used for cloning PCR products for expression of maltose binding protein (MBP) fusion proteins where is also present an N-terminal hexa-histidine tag; obtained from Dr D. I. Roper. **pET33b -** a vector carrying a T7 RNA polymerase promoter and a multiple cloning site. pET33b also carries kanamycin resistance used for cloning PCR products for expression of proteins with a hexa-his tag; obtained from Novagen. **pMW172 -** a vector carrying ampicillin resistance used for cloning PCR products for native expression of proteins (6). **pG-KJE8 -** A vector carrying inducible (by L-Arabinose and tetracycline) chaperones genes as dnaK, dnaJ, grpE, groES, groEL. pG-KJE8 also carry chloramphenicol resistance; obtained from Dr D. I. Roper. **pRARE -** a vector that encode tRNA for the rare in *E. coli* codons AGA, AGG (Arg), GGA (Gly), AUA (Ile),CUA (Leu), CCC (Pro). pRARE also carries chloramphenicol resistant; obtained from Novagen. **pRARE2 -** a vector that encode tRNA for the rare in *E. coli* codons AGA, AGG and CGG (Arg), GGA (Gly), AUA (Ile),CUA (Leu), CCC (Pro). pRARE also carries chloramphenicol resistant; obtained from Novagen.

Supplementary Figure Legends

Figure S1. **Mass spectroscopic analysis of UDP-MurNAc-hexapeptide (L-Ala).** (a) Structures and fragmentation pattern of UDP-MurNAc-pentapeptide and UDP-MurNAc-hexapeptide (L-Ala). The UDP-MurNAc-pentapeptide structure shows the origin of fragment A and B. Fragment C and D are the result of double cleavage event shown in the UDP-MurNAc-hexapeptide (L-Ala) structure. (b) Comparison of positive ion peptide fragmentation spectra of UDP-MurNAc-pentapeptide (top) and UDP-MurNAc-hexapeptide (L-Ala) (bottom). Peptide aminoacyl residues are stated in single letter code: A = L- or D-Ala; E = D-Glu; K = L-Lys; (A) = branched L-Ala attached to ε-NH_2 of L-Lysine. The circled ions (A, B, C and D) confirm that the L-alanine has been attached to the ε-NH_2 of L-lysine of the UDP-MurNAc-pentapeptide. The C ion at *m/z* = 400.23 represents the γ-D-Glu-L-Lys-(L-Ala)-D-Ala that differs from ion A, γ-D-Glu-L-Lys-D-Ala-D-Ala (*m/z* = 418.2570). The ion D at *m/z* = 271.18 represent the L-Lys-(L-Ala)-D-Ala (in brackets the branched L-alanine attached to ε-NH_2 of L-Lys) that differs from ion B, L-Lys-D-Ala-D-Ala (*m/z* = 289.21).

Figure S2. **Mass spectroscopic analysis of UDP-MurNAc-hexapeptide (L-Ser).** (a) Structures and fragmentation pattern of UDP-MurNAc-pentapeptide and UDP-MurNAc-hexapeptide (L-Ser). Structure I shows the origin of fragment A and B. Fragment C and D are the result of double cleavage event shown in structure II. (b) Positive ion MS/MS fragmentation spectra. Peptide aminoacyl residues are stated in single letter code: A = L- or D-Ala; E = D-Glu; K = L-Lys; (S) = branched L-Ser attached to ε-NH_2 of L-Lysine. All the ions containing L-serine are associated with the L-lysine of the UDP-MurNAc-pentapeptide, fragments A-D are an example.

Supplementary Table Legend

Table S1. **Primers used to amplify and sequence murN Pn16 and 159 for cloning into pBADM-41.** The nucleotides in bold type indicate the restriction enzyme site and tags added to the 5' end of the primers. The nucleotides in bold underlined type represent the restriction sites used for cloning. The nucleotides in red type represent the stop codon. Table S1 also shows the MBP-Fw and MBP-Rv primers used to sequence the plasmid insert. The PCR annealing temperature (Temp) used are also shown.

Supplementary References

1. Johnston, T. C., Thompson, R. B., and Baldwin, T. O. (1986) *J Biol Chem* **261**(11), 4805-4811
2. Grant, S. G., Jessee, J., Bloom, F. R., and Hanahan, D. (1990) *Proc Natl Acad Sci U S A* **87**(12), 4645-4649
3. Lopez, P. J., Marchand, I., Joyce, S. A., and Dreyfus, M. (1999) *Mol Microbiol* **33**(1), 188-199
4. Miroux, B., and Walker, J. E. (1996) *Journal of molecular biology* **260**(3), 289-298
5. Leahy, D. J., Hendrickson, W. A., Aukhil, I., and Erickson, H. P. (1992) *Science (New York, N.Y* **258**(5084), 987-991
6. Way, M., Pope, B., Gooch, J., Hawkins, M., and Weeds, A. G. (1990) *The EMBO journal* **9**(12), 4103-4109

Supplementary Figures

Figure S1

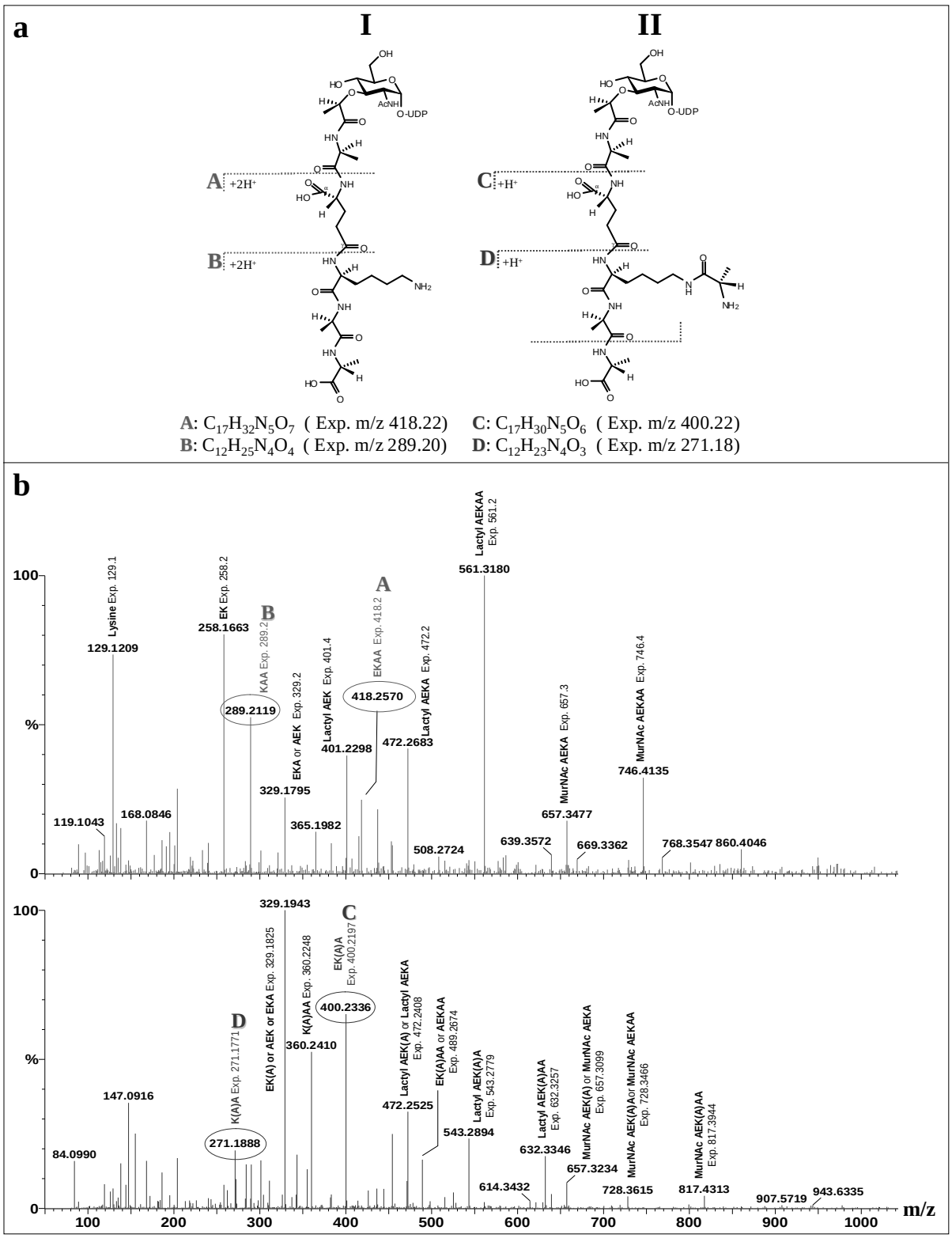
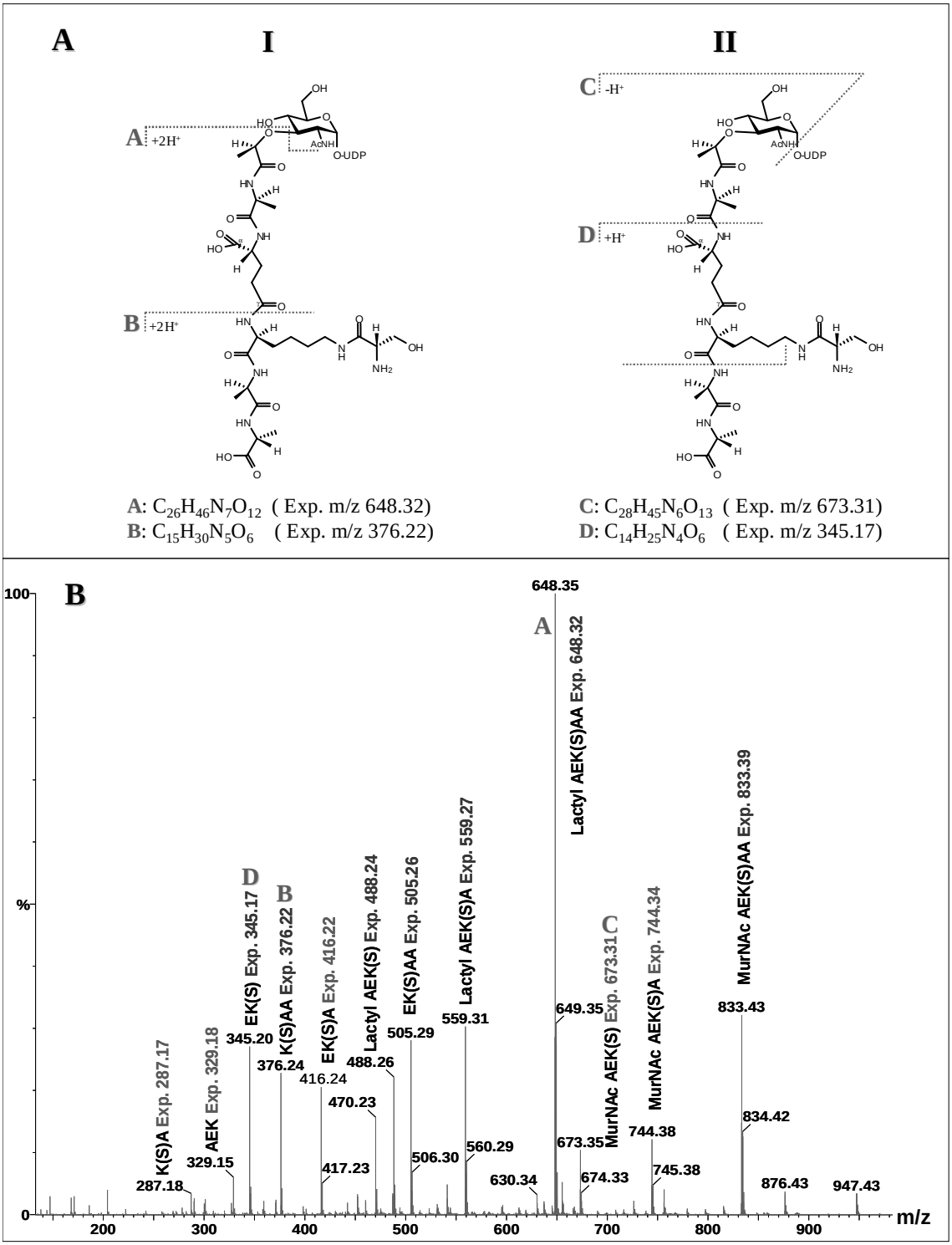


Figure S2


A
I
II
A: +2H+
B: +2H+
C: -H+
D: +H+
O-UDP
AcNH
A: C26H46N7O12 (Exp. m/z 648.32)
B: C15H30N5O6 (Exp. m/z 376.22)
C: C28H45N6O13 (Exp. m/z 673.31)
D: C14H25N4O6 (Exp. m/z 345.17)
B
100
%
0
m/z
200
300
400
500
600
700
800
900
648.35
A
Lactyl AEK(S)AA Exp. 648.32
649.35
MurNAc AEK(S) Exp. 673.31
C
673.35
674.33
MurNAc AEK(S)A Exp. 744.34
744.38
745.38
MurNAc AEK(S)AA Exp. 833.39
833.43
834.42
876.43
947.43
Lactyl AEK(S)A Exp. 559.27
559.31
560.29
630.34
EK(S)AA Exp. 505.26
505.29
506.30
Lactyl AEK(S) Exp. 488.24
488.26
470.23
EK(S)A Exp. 416.22
416.24
417.23
K(S)AA Exp. 376.22
B
376.24
329.15
EK(S) Exp. 345.17
D
345.20
AEK Exp. 329.18
K(S)A Exp. 287.17
287.18

Supplementary Table

Table S1

Name of Primer	**Primer Sequence**	**PCR Anneal Temp (°C)**
murN-Nco1 Fw	5' **CATGCC*ATGG***CACTAACAACACTCA 3'	50
MBP-murM-Xho1 Rv	5' **CCCGCTCGAG**TAAACGTCCTACTATTTTTTTG 3'	50
MBP Fw	5' CTGAAAGACGCGCAGACTAATC 3'	55
MBP Rv	5' CAGACCGCTTCTGCGTTCTG 3'	55