Solid State NMR studies of intact lipopolysaccharide endotoxin

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Supplementary Table 1

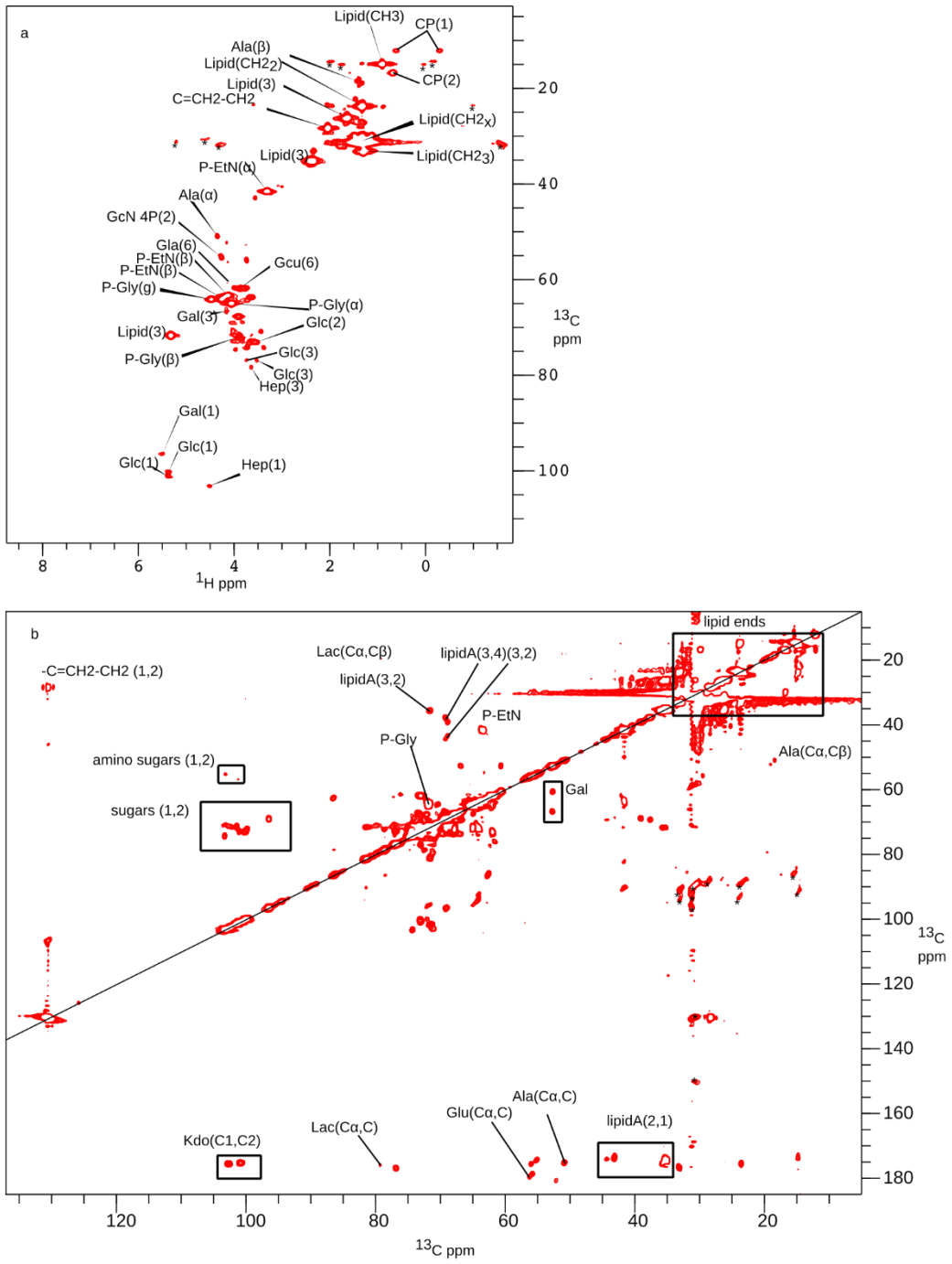
		C1	C2	C3	C4	C5	C6	H1	H2	H3	H4	H5	H6
Lipid A	**6-α-GlcN 1P**	95,0	53,4	74,5									
Lipid A	**6-α-GlcN 1P**	95,7	53,2	74,4									
Lipid A	**6-β-GlcN 4P**	103,2	55,3	75,0			64,4		4,26				
Core	**α-Kdo**	175,6	102,7										
Core	**α-Kdo**	175,3	100,8										
Core	**3-α-Hep 4-P**	99,9	72,3	78,6	74,7					3,644			
Core	**3,7 -α-Hep 4P**	101,5	71,5	81,2									
Core	**3-α-Glc**	100,3	73,1	77,0	74,4			5,38	3,53				
Core	**t-α-Gal**	96,5	69,3	66,9	66,9	52,7	60,6	5,49		4,15		3,74	3.84/4.14
Core	**3-αGlc***	101,1	73,1										
Core	**2-α-Glc***	103,3	74,3	70,8				4,51					
Core	**t-α-Rha***	102,3	71,5										
		C1	**C2**	**C3**	**C4**	**C5**	**C6**		**H1**	**H2**	**H3**	**H4**	
Lipid A	**C=O-CH2-COH/OR-CH2-CH2-CH2x**	174,3	44,4	69,3	37,7	27,4	31,6						
Lipid A	**C=O-CH2-COH/OR-CH2-CH2-CH2x**	173,5	43,1	68,8	39,0	27,0	31,6			4,06			
Lipid A	**C=O-CH2-COH/OR-CH2-CH2-CH2x**	175,5	35,7	71,9	35,5	26,6	31,3			2,38	5,31		
Lipid	**C=O-CH2-CH2-CH2x**	174,4	35,2	26,2	31,0					2,35	1,63		
Lipid	**C=C-CH2-CH2x-**	130,6	28,4	31,1					5,33	2,03	1,32		
Lipid	**Cyclopropane**	12,1	16,8	29,9	31,3				0.61/-0.30	0,68	1,48	1,13	
		CH_3	**$CH2_1$**	**$CH2_2$**	**$CH2_x$**			**CH_3**	**$CH2_1$**	**$CH2_2$**	**$CH2_x$**		
Lipid A	**CH3-CH2-CH2-CH2x-**	14,8	23,7	33,1	31,0			0,89	1,31	1,28	1,34		
		Cα	**Cβ**	**Cγ**			**Hα**	**Hβ**	**Hγ**				
P-Etn	**P-O-CH2-CH2-NH3+**	41,6	63,9				3,31	4,24					
P-Etn	**P-O-CH2-CH2-NH3+**	41,8	63,4				3,30	4,13					
P-Gly	**P-O-CH2-CHOH-CH2-OH**	65,1	71,9	64,3			4,05	3,91	4,47				
		C1	**C2**	**C3**	**C4**	**C5**	**C6**	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**
PG	**β-MurNac**	101,3	56,9	81,0	73,6	76,0	61,7						
PG	**β-MurNac**	102,5	56,3	80,8	73,5	76,2	61,7	4,13				3,88	
PG	**β-GlcNac**	101,1	56,8	73,6									
		C	**Cα**	**Cβ**				**Hα**	**Hβ**				
PG	**Alanine**	175,1	50,9	18,4				4,34	1,43				
PG	**Lactoyl**	176,0	79,5	19,5									
PG	**Glutamate**	178,7	55,8	29,5									

Escherichia coli K12 LPS ^{13}C and ^{1}H chemical shift (ppm) assigned from solid state NMR experiments.
PG (peptidoglycan). Underlined chemical groups are the ones for which the assignment is given. CH2x accounts for CH2s constituting the middle of the lipid chains and that are all chemically equivalent. *Putative assignments

Supplementary Table 2

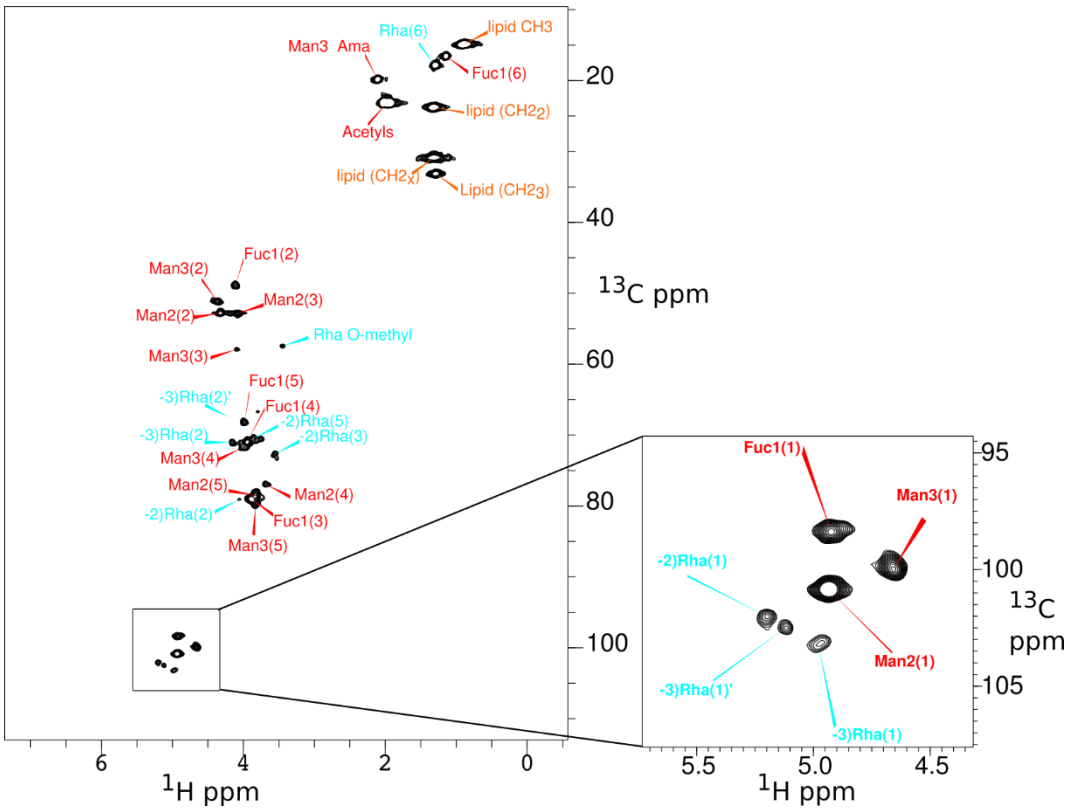
		CH_3	$CH2_1$	$CH2_2$	$CH2_x$			CH_3	$CH2_1$	$CH2_2$	$CH2_x$			
Lipid A	**CH3-CH2-CH2-CH2x-**	14,9	23,9	33,3	31,0			0,90	1,32	1,29	1,31			
		Cα	**Cβ**											
Lipid	**C=C-CH2-**	130,4	28,2											
		C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**
core	**α-Kdo**	176,3		35,4	73,1									
core	**3)-α-Hep(1-5**	105,3	74,6	76,8	70,6	77,0		61,8						
core	**3)-α-Rha**	101,2	71,2	81,2	73,8	70,2	18,1					3,49		
		Cα	**Cβ**											
core	**Alanine**	50,5	18,1											
core	**Alanine**	51,5	17,9											
A-Band	**2)-α-Rha**	101,9	79,3	73,7	71,1	70,4	17,8		5,20	4,06	3,55		3,86	1,30
A-Band	**3)-α-Rha**	103,3	71,1						4,98	4,16				
A-Band	**3)-α-Rha'**	102,5	67,4						5,12	4,20				
A-Band O-CH3	**Rha**	57,4							3,45					
O-Acetyl		21,3	174,6						2,15					
		C1	**C2**	**C3**	**C4**	**C5**	**C6**		**H1**	**H2**	**H3**	**H4**	**H5**	**H6**
B-Band	**α-Fuc1**	98,4	48,7	79,1	70,9	68,1	16,5		4,92	4,12	3,89	3,95	3,98	1,14
B-Band	**β-Man2**	100,9	52,7	52,8	77,0	78,2	176,4		4,93	4,32	4,08	3,68	3,83	
B-Band	**β-Man3**	100,0	51,1	57,8	71,7	79,9	175,3		4,66	4,36	4,10	4,02	3,83	
		CH3-C=O	**CH3-C=O**	**CH3-C=N**	**CH3-C=N**				**CH3-C=O 2/3**	**CH3-C=N**				
B-Band	**α-Fuc1**	175.3/176.1a	23,1						1,94					
B-Band	**β-Man2**	175.3/176.1a	23,1						1.942/2.023					
B-Band	**β-Man3**	175.3/176.1a	23,1	167,3	19,8				1,94	2,11				

Pseudomonas Aeruginosa PAO1 LPS ^{13}C and ^{1}H chemical shifts (ppm) obtained from solid state NMR experiments. t: terminal sugar (glycosydic bond only involving anomeric group)
a: ambiguous value



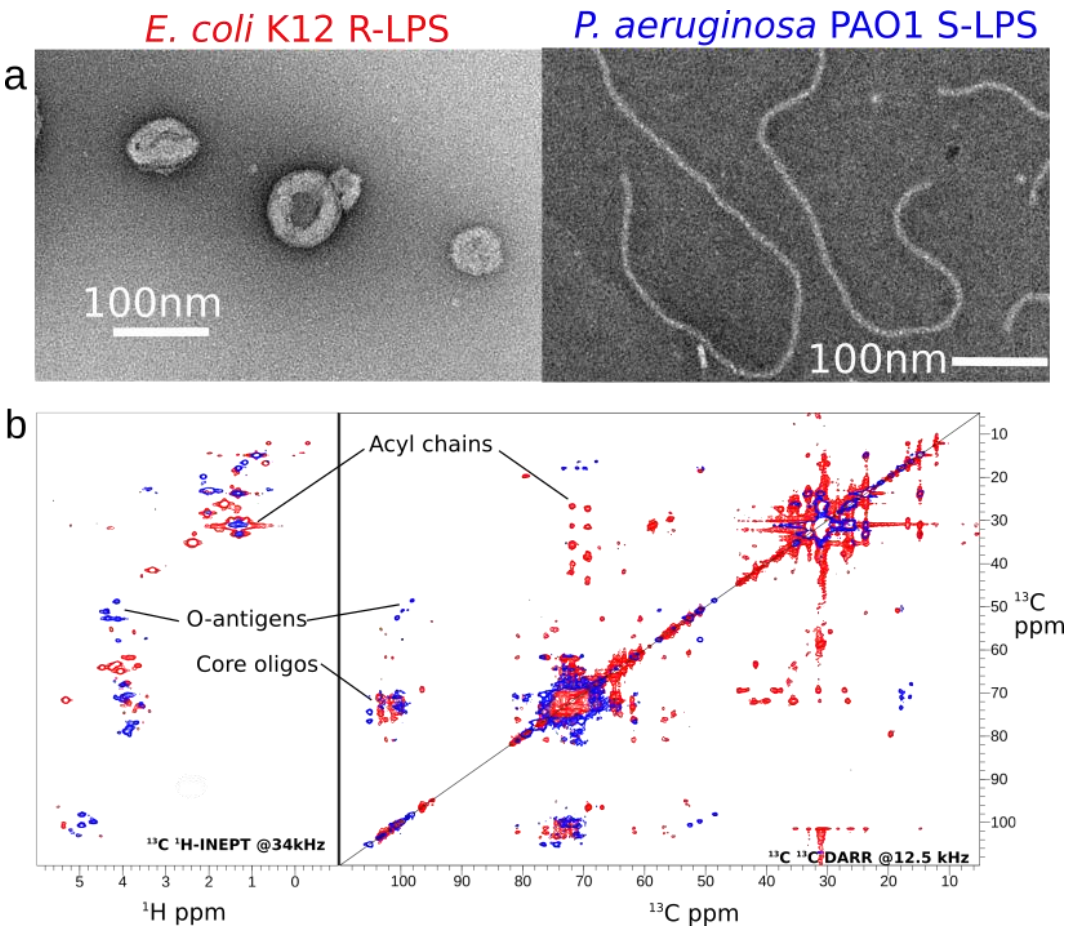
Supplementary Figure 1

(a) ^{13}C-^{1}H-INEPT spectrum of *E. coli* R-LPS at 34kHz. (b)^{13}C-^{13}C Single Quantum J-correlation experiment of *E. coli* R. LPS at 12.5kHz. Asterisks mark a diagonal of artefacts on the spectra. CP: cyclopropane.



Supplementary Figure 2

^{13}C-^{1}H-INEPT spectrum of *P. aeruginosa* PAO1 S-LPS at 34kHz. Assignments labels are colored orange, red and cyan for lipidA, B-band and A-band parts, respectively.

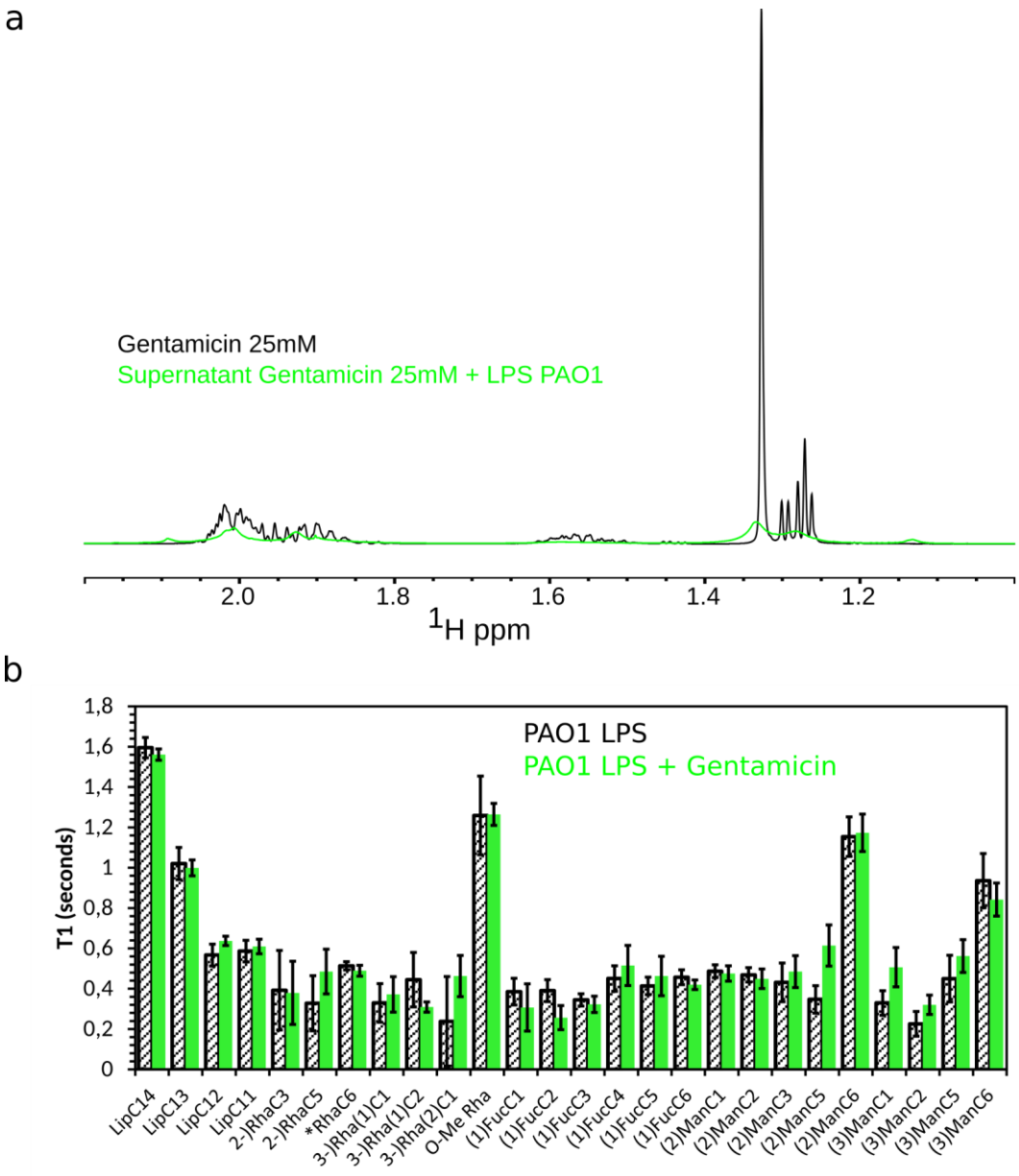


Supplementary Figure 3:

LPS from *E. coli* and *P. aeruginosa* EM and ssNMR comparison

a) Negative stain Electron micrographs (23000x magnification) of *E. coli* LPS (left) that forms mainly large vesicles in solution and *P. aeruginosa* LPS (right) which forms structures corresponding to elongated micelles.

b) Overlay of *E. coli* (red) and *P. aeruginosa* (blue) ssNMR spectra with ^{13}C-^{1}H-INEPT on the left and ^{13}C-^{13}C DARR on the right.

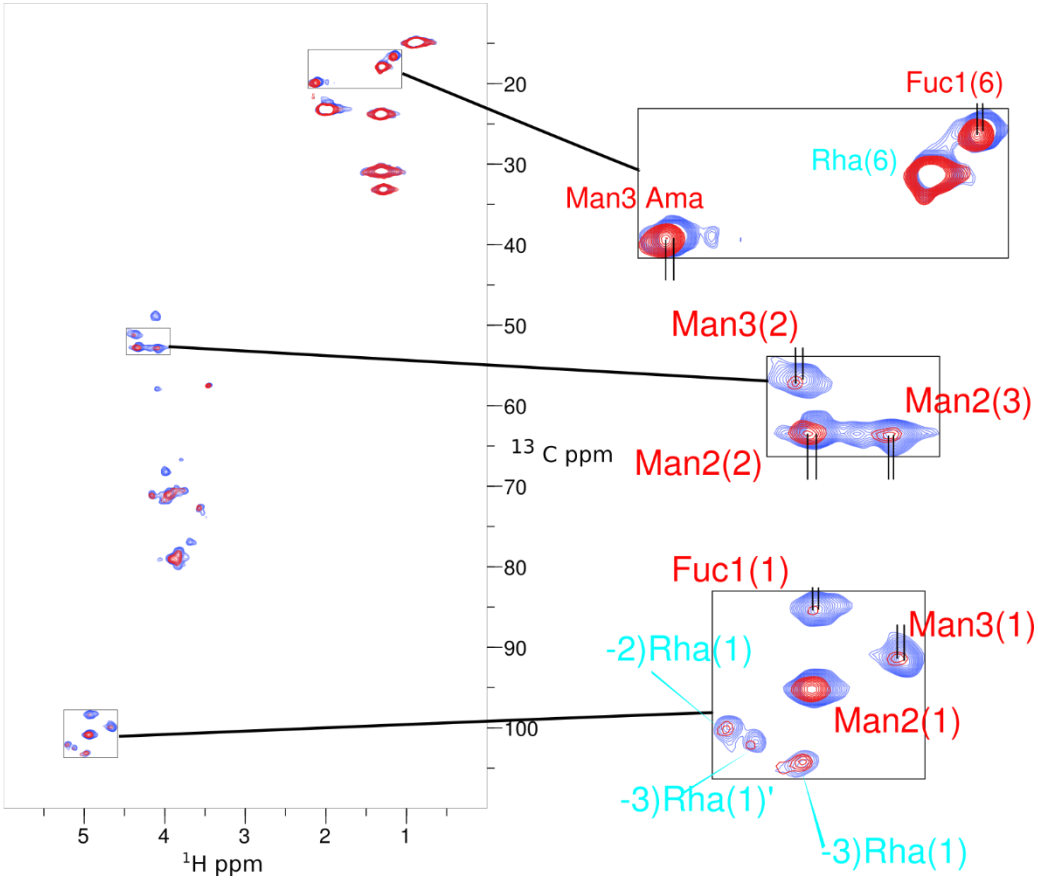


Supplementary Figure 4

LPS PAO1 interacts strongly with gentamicin antibiotic

(a) 1D ^{1}H solution NMR spectrum of 25mM gentamicin (black). PAO1 LPS is added to the gentamicin and solution is ultracentrifuged into the NMR rotor. The ^{1}H solution 1D spectrum of the supernatant (green) displays only broad NMR signal.

(b) T_1 relaxation time constants of PAO1 LPS in absence (hatched) and presence (green) of gentamicin.



Supplementary Figure 5

^{13}C-^{1}H INEPT ssNMR spectrum of *P. aeruginosa* LPS in absence or presence (red) of 25mM gentamicin. Spectra are normalized to the intensity of lipid CH_3 peak. Several regions of the spectra are enlarged (x4) to visualize chemical shift variations of LPS induced by gentamicin. Vertical bars were drawn to highlight ^{1}H chemical shift induced by gentamicin.