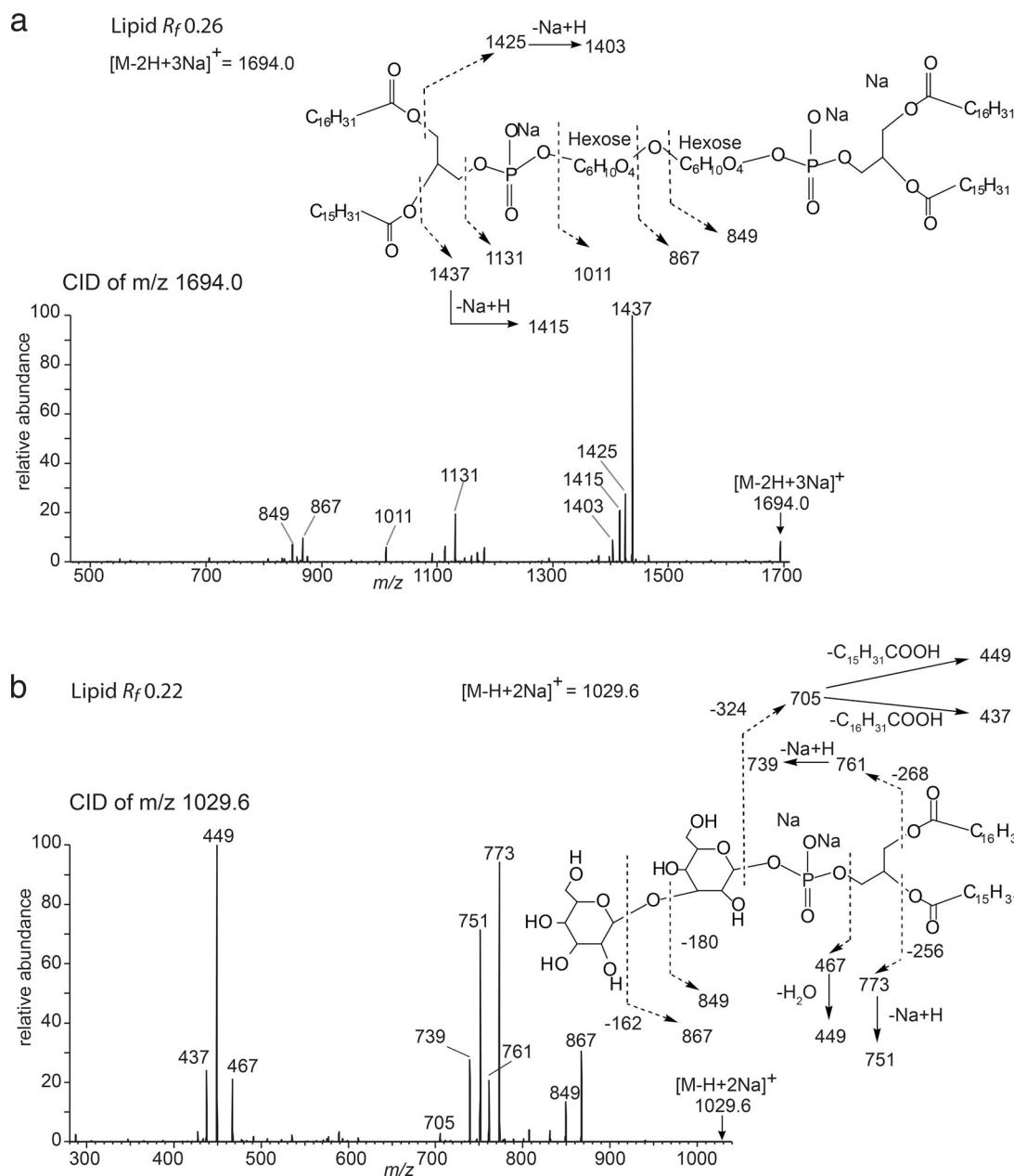


Supplemental material

Reinink et al., <https://doi.org/10.1084/jem.20181812>



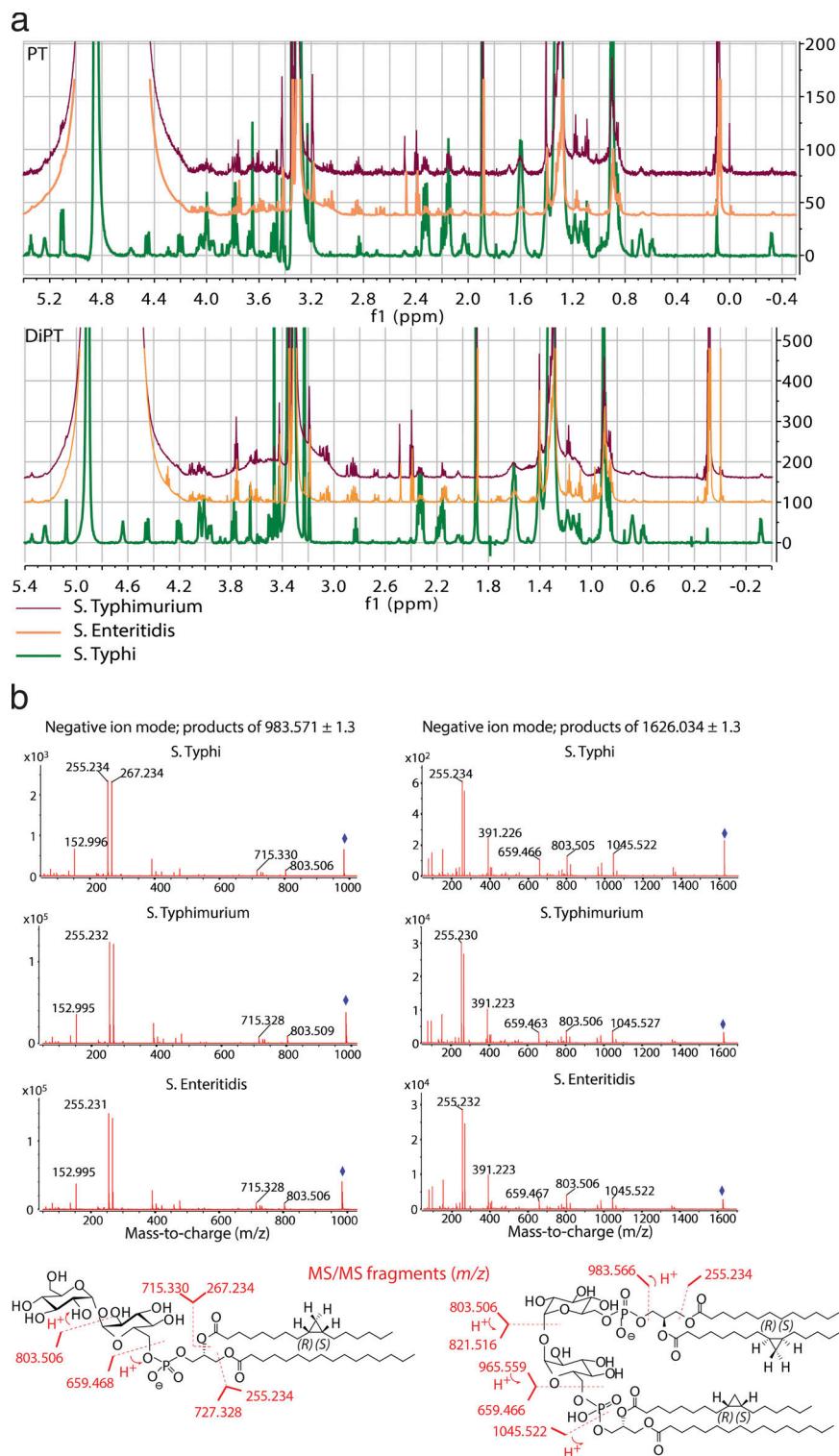


Figure S2. Comparison of PT and diPT from *S. Typhi*, *S. Enteritidis*, and *S. Typhimurium*. (a) ^1H -NMR spectra of TLC-isolated PT (upper panel) and diPT (lower panel) from *S. Typhi*, *S. Enteritidis*, and *S. Typhimurium* were merged and aligned. (b) Negative ion mode high-accuracy collisional mass spectra of TLC-isolated PT (left panels) and diPT (right panels) from *S. Typhi*, *S. Enteritidis*, and *S. Typhimurium*.

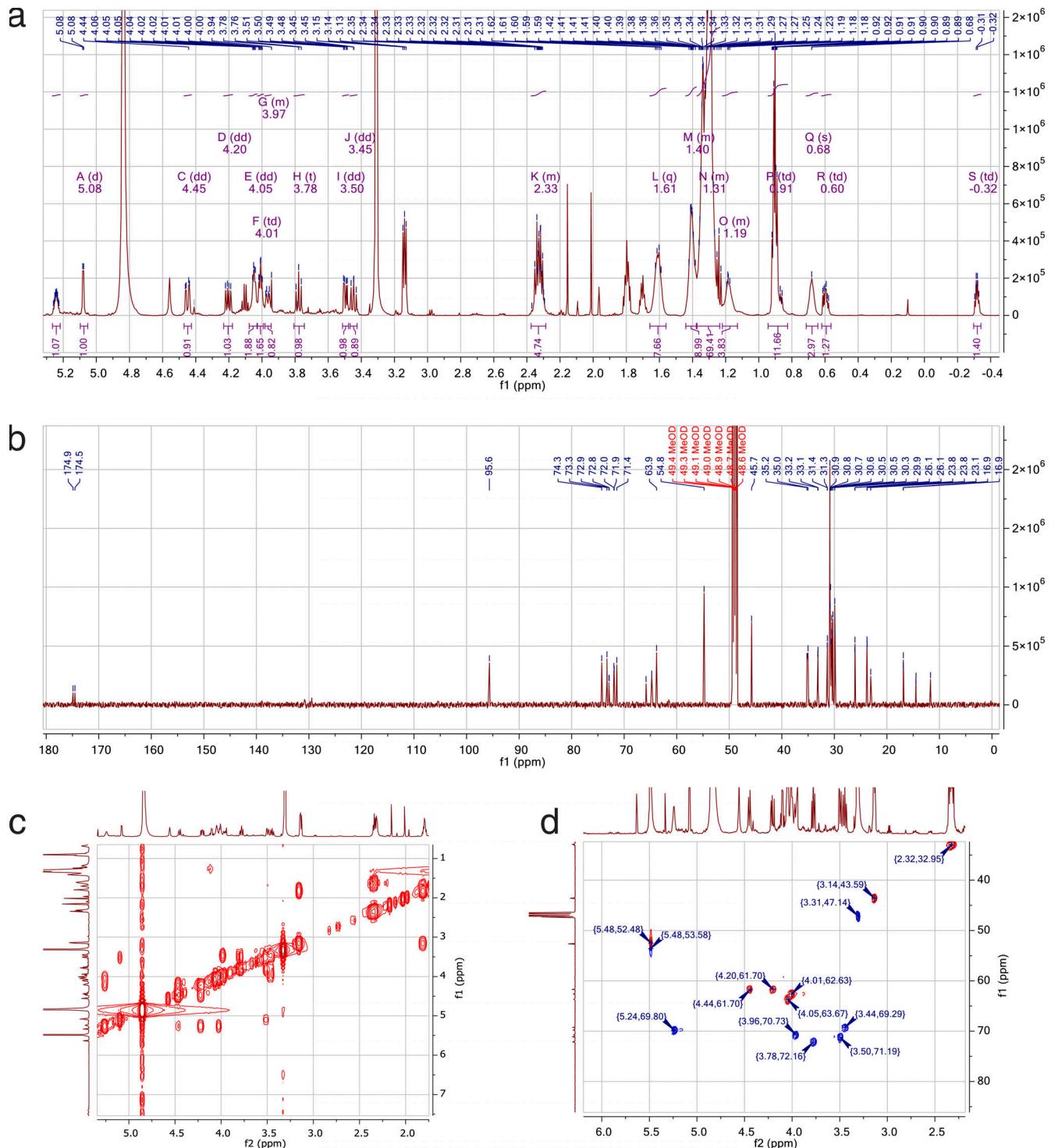


Figure S3. NMR of synthetic diPT. (a-d) Synthetic diPT was analyzed with ^1H -NMR (600 MHz; a), ^{13}C -NMR (150 MHz; b), gradient-selected COSY (c), and multiplicity-edited HSQC ^1H -NMR (600 MHz, CD3OD; d): 5.24 (dtd, $J = 6.7, 5.1, 3.1$ Hz, 1H), 5.08 (d, $J = 3.8$ Hz, 1H), 4.45 (dd, $J = 12.0, 3.1$ Hz, 1H), 4.20 (dd, $J = 12.0, 6.9$ Hz, 1H), 4.05 (dd, $J = 6.2, 3.5$ Hz, 2H), 4.01 (td, $J = 5.5, 1.6$ Hz, 2H), 3.98–3.94 (m, 1H), 3.78 (t, $J = 9.4$ Hz, 1H), 3.50 (dd, $J = 9.7, 3.8$ Hz, 1H), 3.45 (dd, $J = 10.0, 9.0$ Hz, 1H), 2.37–2.29 (m, 4H), 1.61 (q, $J = 6.9, 6.2$ Hz, 7H), 1.44–1.38 (m, 8H), 1.37–1.24 (m, 65H), 1.22–1.13 (m, 4H), 0.91 (td, $J = 7.0, 4.2$ Hz, 11H), 0.68 (s, 3H), 0.60 (td, $J = 8.3, 4.1$ Hz, 1H), -0.32 (td, $J = 5.4, 4.1$ Hz, 1H). ^{13}C -NMR (150 MHz, MeOD): 174.9, 174.539, 95.7, 74.3, 73.3, 72.9, 72.9, 71.96, 71.9, 71.4, 65.8, 65.8, 64.9, 64.75, 63.9, 54.8, 45.7, 35.2, 35.0, 33.2, 33.1, 31.4, 31.3, 30.9, 30.8, 30.7, 30.6, 30.5, 30.5, 30.3, 29.9, 26.1, 26.1, 23.8, 23.8, 23.1, 16.9, 16.9, 14.5, 14.5, 11.7.

Table S1. Abundance of PT, diPT, PE, and CL in *S. Typhimurium* single gene knockouts

Gene mutated	m/z 983 (PT)	m/z 1,626 (diPT)	m/z 647 (PE)	m/z 1,389 (CL)
Kanamycin-selected mutants peak area ($\times 10^3$)				
Aas	1,202	100	1,289	1,533
ClmA	1,147	121	1,632	1,366
ClmB	0	0	1,827	973
EntF	723	64	1,919	1,296
OtsA	214	23	1,731	1,651
OtsB	278	56	1,957	1,652
PagP	567	47	1,831	1,120
PgpA	1,274	104	1,677	1,453
PgpB	932	103	1,733	1,586
PgpC	661	48	1,919	1,581
TreY	349	28	2,072	1,219
TreZ	833	87	2,215	1,417
WT	467	48	1,740	1,360
Chloramphenicol-selected mutants peak area ($\times 10^3$)				
Aas	392	33	718	636
ClmA	522	46	818	331
ClmB	0	0	728	581
EntF	560	51	919	724
OtsA	631	57	906	660
OtsB	42	7.6	933	623
PagP	278	25	791	701
PgpA	119	11	904	880
PgpB	316	31	891	668
PgpC	593	50	716	379
TreY	482	31	1,052	794
TreZ	566	53	789	680
WT	36	6.9	972	657

Total lipid extracts from the indicated *S. Typhimurium* single gene knockouts were analyzed by reverse-phase HPLC-MS. Areas under the indicated extracted ion chromatograms are shown for PT (m/z 983.5714 \pm 10 ppm), diPT (1,626.0338 \pm 10 ppm), PE (674.4770 \pm 10 ppm), and CL (1,389.9806 \pm 10 ppm).

Table S2. Bacterial strains and culture conditions

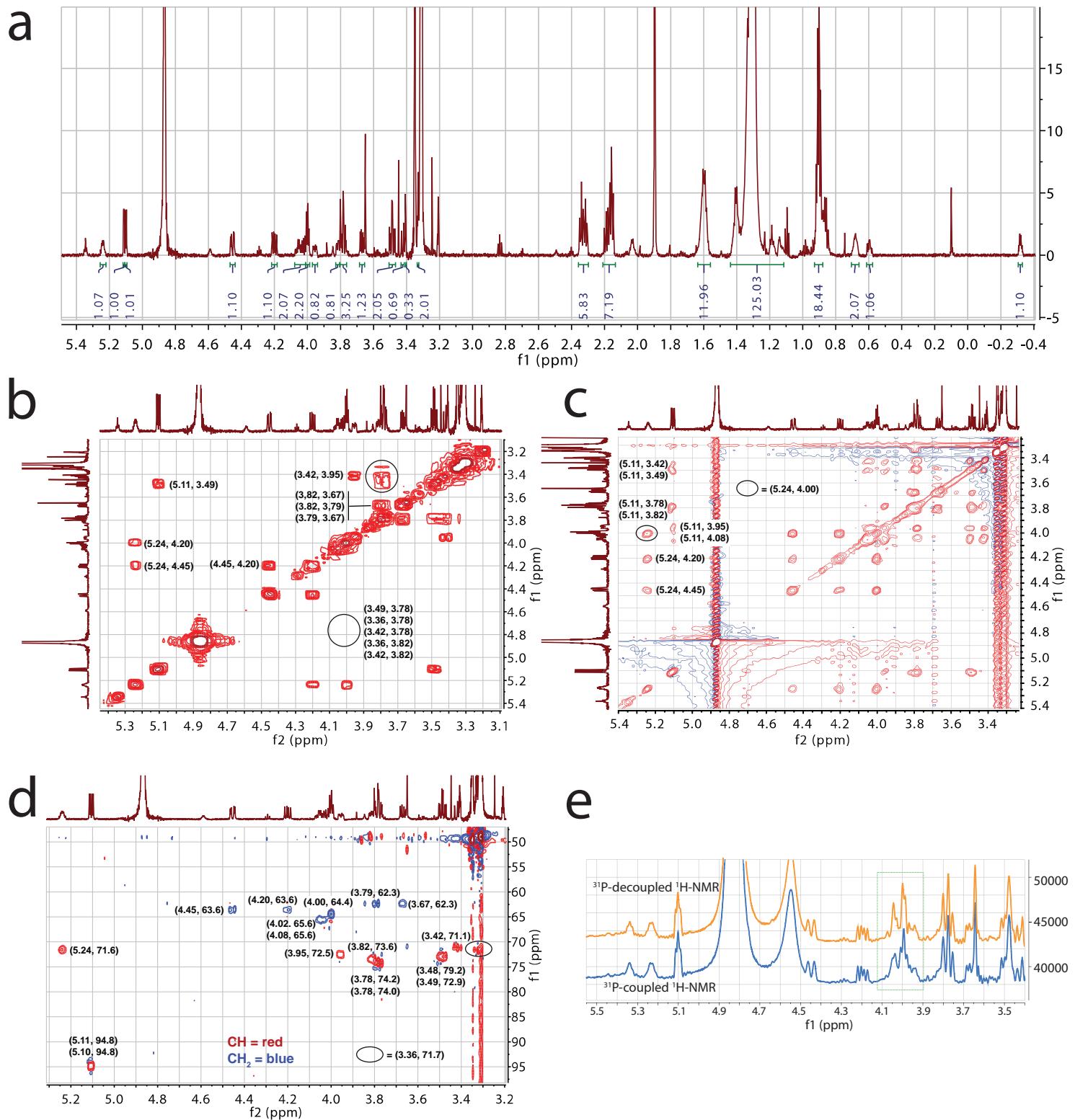
Species or strain	Identifier	Culture medium
S. Enteritidis	SAL RIVM 423.78	LB
S. Enteritidis	SAL RIVM 423.79	LB
S. Enteritidis	SAL RIVM 423.68	LB
S. Paratyphi B	SAL RIVM 423.37	LB
S. Paratyphi A	SAL RIVM 422.26	LB
S. Paratyphi A	SAL RIVM 421.73	LB
S. Paratyphi A	NVGH 308	LB
S. Typhi	SAL RIVM 424.47	LB
S. Typhi	SAL RIVM 422.41	LB
S. Typhi	SAL RIVM 421.56	LB
S. Typhi	Quailes	LB
S. Typhimurium	SAL RIVM 423.77	LB
S. Typhimurium	SAL RIVM 423.57	LB
S. Typhimurium	SAL RIVM 423.62	LB
S. Typhimurium	14028s	LB
Salmonella bongori	RIVM 66:Z65	LB
Shigella dysenteriae	BD09-OO284, serovar 3	LB
Shigella flexneri	BD09-0271	LB
E. coli	DH10B	LB
E. coli	DH5a	LB
E. coli	bw25113	LB
E. coli	ATCC 25922	LB
E. coli	EHEC CVI-7, O150:H2	LB
E. coli	EHEC CVI-19, O177:H25	LB
P. aeruginosa	ATCC 27853	LB
P. aeruginosa	NCTC 10662	LB
Brucella melitensis	x10017283-001 (WBVR)	Tryptic soy broth
B. pertussis	B1917	THIJS (Thalen et al., 1999)
M. tuberculosis	H37Ra	Obtained dry (Difco)
Rhodococcus equi	ATCC 33701	Brain heart infusion broth
Staphylococcus aureus	Coagulase+ clinical isolate	Brain heart infusion broth
Lactobacillus johnsonii	Cecum of BALB/c mouse	Basal medium, anaerobic (Zitomersky et al., 2011)
Bacteroides ovatus	ATCC 8483	Basal medium, anaerobic (Zitomersky et al., 2011)
Bacteroides vulgatus	ATCC 8482	Basal medium, anaerobic (Zitomersky et al., 2011)
Bacteroides thetaiotaomicron	VPI 5482	Basal medium, anaerobic (Zitomersky et al., 2011)
Bacteroides fragilis	NCTC 9343	Basal medium, anaerobic (Zitomersky et al., 2011)

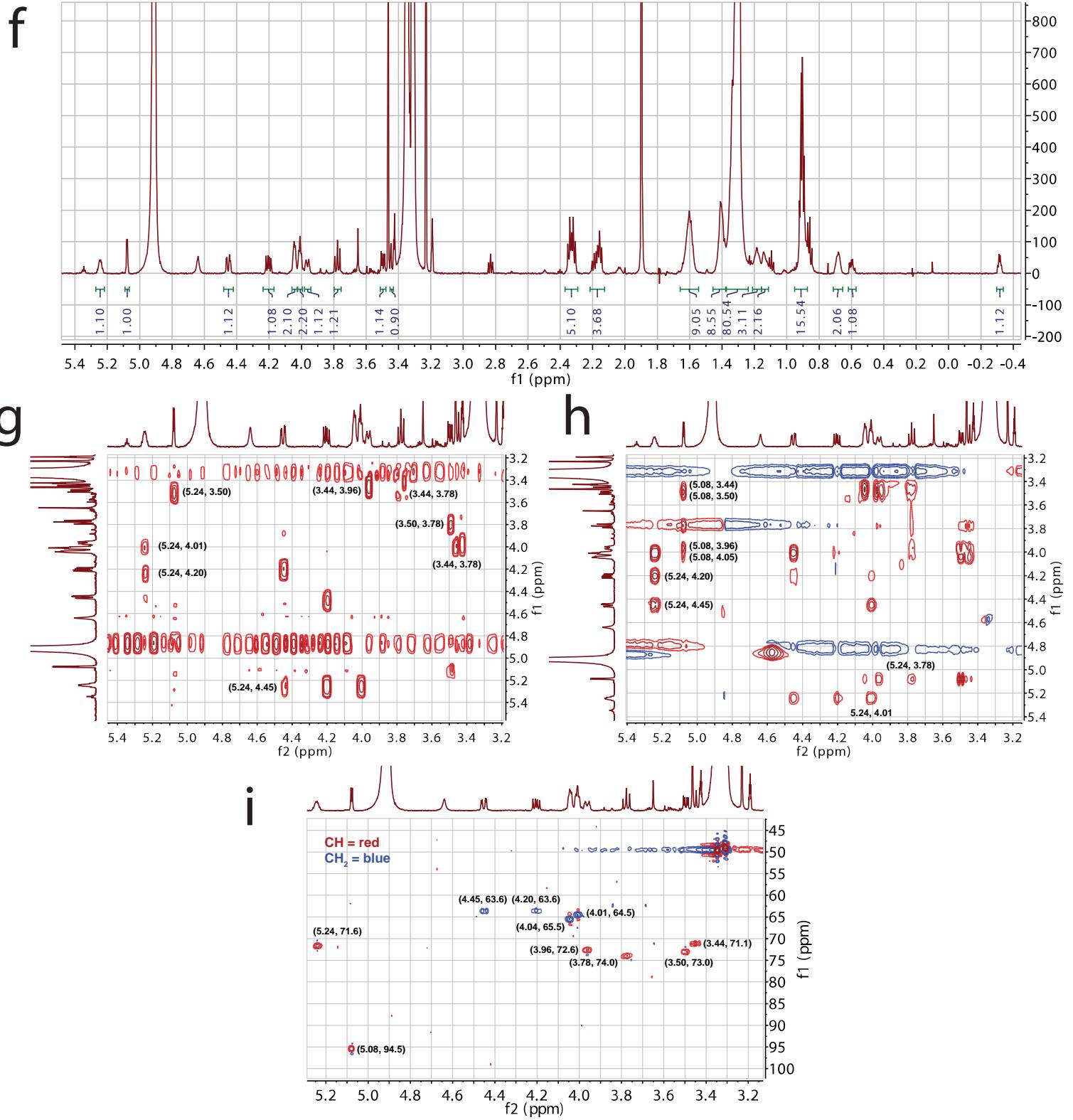
RIVM, Rijksinstituut voor Volksgezondheid en Milieu; ATCC, American Type Culture Collection; NCTC, National Collection of Type Cultures; NVGH, Novartis Vaccines Institute for Global Health; EHEC, enterohemorrhagic *E. coli*.

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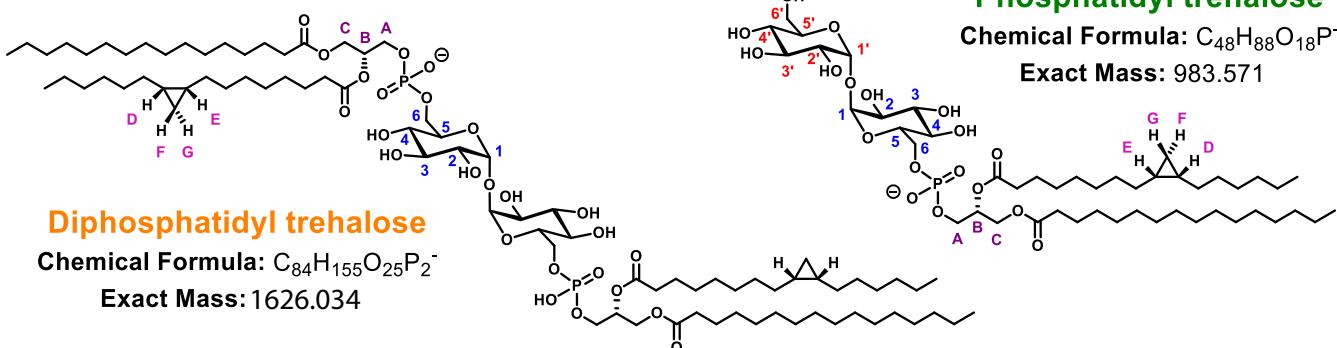
Dataset 1





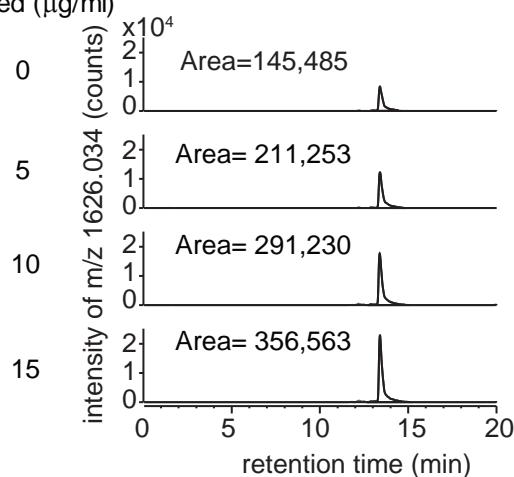
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Salmonella Typhi trehalose phospholipids

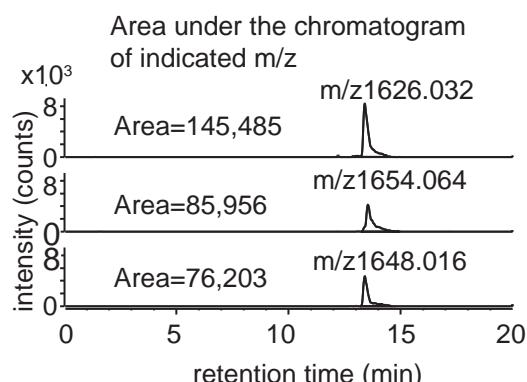
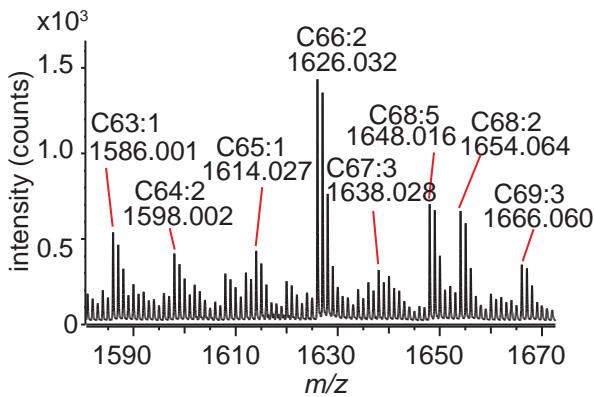
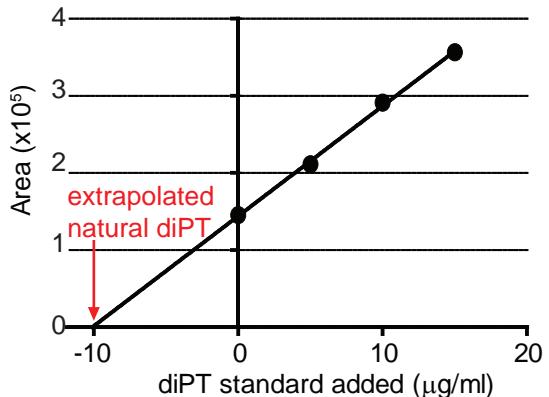


	Phosphatidyl trehalose				Diphosphatidyl trehalose				
Position	multiplicity	¹ H (ppm)	<i>J</i> _{H,H} (Hz)	¹³ C (ppm) ^a	multiplicity	¹ H (ppm)	<i>J</i> _{H,H} (Hz)	¹³ C (ppm) ^a	
1	d	5.11	3.7	94.8	d	5.08	3.7	94.5	
2	dd ^b	3.49	3.8, 9.8	72.9	dd	3.50	3.6, 9.7	73.0	
3	dd ^c	3.78	9.7	74.0	t	3.78	9.4	74.0	
4	dd ^d	3.42	9.4	71.1	d	3.44	9.9	71.1	
5	m	3.94-3.96		72.5	dt	3.96	2.0, 9.2	72.6	
6a	m	4.02		65.6	m	4.02-4.06		65.5	
6b	ddd ^c	4.08	2.2, 5.6*, 11.6	see 6a	m	see 6a		see 6a	
1'	d	5.10	3.7	94.8					
2'	dd ^b	3.48	3.8, 9.8	72.9					
3'	dd ^c	3.78	9.7	74.2					
4'	m	3.36		71.7					
5'	ddd	3.82	2.3, 5.3, 10.3	73.6					
6'a	m	3.76-3.81		62.3					
6'b	dd	3.67	5.2, 11.8	see 6'a					
A	t	4.00	5.6	64.4	t ^c	4.01	5.7	64.5	
B	m	5.21-5.27		71.6	m	5.22-5.27		71.6	
C1	dd	4.45	3.0, 12.1	63.6	dd	4.45	3.0, 12.0	63.6	
C2	dd	4.20	6.9, 12.0	see C1	dd	4.20	6.9, 12.0	see C1	
D	m	0.66-0.70		16.6	m	0.65-0.68		16.5	
E	m	0.66-0.70		16.6	m	0.65-0.68		16.5	
F_{cis}	ddd	0.60	4.3		ddd	0.60	3.7, 4.5		
G_{trans}	q	-0.32	5.0		q	-0.32	5.0		
C=O^e				173.2					

K

diPT standard
added ($\mu\text{g/ml}$)

diPT quantification by HPLC-MS

estimated amount ($\mu\text{g/ml}$)S. Typhi total lipids
injected at 1 mg/ml

	m/z 1626 (C66:2) with the spiked-in synthetic standard	m/z 1654 (C68:2) area relative to m/z 1626	m/z 1648 (C68:5) area relative to m/z 1626	sum of 3 chain length variants ($\mu\text{g/ml}$)	% of diPT in S. Typhi total lipids
Sample 1	8.35 $\mu\text{g/ml}$	3.25 $\mu\text{g/ml}$	3.44 $\mu\text{g/ml}$	15.04 $\mu\text{g/ml}$	1.5 %
Sample 2	10.1 $\mu\text{g/ml}$	4.32 $\mu\text{g/ml}$	3.88 $\mu\text{g/ml}$	18.3 $\mu\text{g/ml}$	1.8 %
Sample 3	23.4 $\mu\text{g/ml}$	9.7 $\mu\text{g/ml}$	8.4 $\mu\text{g/ml}$	41.5 $\mu\text{g/ml}$	4.2 %

Dataset 1. **Identification of phosphatidyltrehalose and diphosphatidyltrehalose from *S. Typhi* by NMR. (a–e)** Identification of phosphatidyltrehalose from *S. Typhi* by NMR. The TLC-isolated lower (*Rf* 0.22) band lipid from *S. Typhi* was analyzed by ¹H-NMR (a), COSY (b), and TOCSY (c) with 100-ms spin lock, 135DEPT-HSQC (d), and ³¹P-coupled and ³¹P-decoupled ¹H-NMR (e). ¹H-NMR (700 MHz, MeOH-*d*4): δ 5.27–5.21 (m, 1H), 5.11 (d, *J* = 3.7 Hz, 1H), 5.10 (d, *J* = 3.7 Hz, 1H), 4.45 (dd, *J* = 12.1, 3.0 Hz, 1H), 4.20 (dd, *J* = 12.0, 6.9 Hz, 1H), 4.05 (ddd, *J* = 11.8, 5.6, 2.2 Hz, 2H), 4.00 (t, *J* = 5.6 Hz, 2H), 3.98–3.94 (m, 1H), 3.82 (ddd, *J* = 10.3, 5.3, 2.3 Hz, 1H), 3.80–3.76 (m, 3H), 3.67 (dd, *J* = 11.8, 5.2 Hz, 1H), 3.49 (td, *J* = 9.8, 3.8 Hz, 2H), 3.41 (dd, *J* = 9.4 Hz, 1H), 3.34–3.33 (m, 1H), 2.37–2.29 (m, 6H), 2.21–2.13 (m, 7H), 1.66–1.55 (m, 12H), 1.44–1.12 (m, 55H), 0.92–0.88 (m, 13H), 0.70–0.66 (m, 2H), 0.60 (ddd, *J* = 4.3 Hz, 1H), –0.32 (q, *J* = 5.0 Hz, 1H). ¹³C (176 MHz, MeOH-*d*4) – signals observed in the multiplicity-edited HSQC: δ 94.8, 74.2, 74.0, 73.6, 72.9, 72.5, 71.6, 71.1, 65.6, 64.4, 63.6, 62.3, 38.7, 36.3, 34.7, 32.6–23.6 (multiple resonances), 27.3, 25.8, 22.6, 19.9, 16.6, 14.5. **(f–i)** Identification of diphosphatidyltrehalose from *S. Typhi* by NMR. The TLC-isolated upper (*Rf* 0.26) band lipid from *S. Typhi* was analyzed by ¹H-NMR (f), COSY (g), and TOCSY (h) with 100-ms spin lock and 135DEPT-HSQC (i). ¹H-NMR (600 MHz, MeOH-*d*4): δ 5.27–5.22 (m, 1H), 5.08 (d, *J* = 3.7 Hz, 1H), 4.45 (dd, *J* = 12.0, 3.0 Hz, 1H), 4.20 (dd, *J* = 12.0, 6.9 Hz, 1H), 4.06–4.02 (m, 2H), 4.01 (t, *J* = 5.7 Hz, 2H), 3.96 (dt, *J* = 9.2, 2.0 Hz, 1H), 3.78 (t, *J* = 9.4 Hz, 1H), 3.50 (dd, *J* = 9.7, 3.6 Hz, 1H), 3.44 (d, *J* = 9.9 Hz, 1H), 2.37–2.29 (m, 5H), 2.22–2.12 (m, 4H), 1.66–1.55 (m, 9H), 1.40 (d, *J* = 7.6 Hz, 8H), 1.37–1.24 (m, 80H), 1.19 (dd, *J* = 8.5, 4.6 Hz, 3H), 1.14 (s, 2H), 0.91 (td, *J* = 7.0, 4.5 Hz, 16H), 0.71–0.65 (m, 2H), 0.60 (ddd, *J* = 4.5, 3.7 Hz, 1H), –0.32 (q, *J* = 5.0 Hz, 1H). ¹³C signals (151 MHz, MeOH-*d*4) observed in the multiplicity-edited HSQC: δ 95.4, 74.0, 73.0, 72.6, 71.6, 71.1, 65.5, 64.5, 63.6, 38.8, 36.4, 34.8, 32.9–23.5 (multiple resonances), 27.5, 25.9, 22.9, 19.8, 16.6, 14.3, 11.6. **(j)** Summary table of the correlation of NMR data with structure. NMR assignments of the lower and upper band isolated from *S. Typhi*. a, assigned using the multiplicity-edited HSQC spectrum; b, apparent doublet; c, apparent multiplet; d, apparent triplet; e, assigned using a heteronuclear multiple bond correlation spectrum; *, ³JH,P coupling; gray boxes, not applicable or not obtained. **(k)** *S. Typhi* total lipids were spiked with known concentrations of synthetic diPT (C66:2 with *m/z* 1,626.032) and subjected to reverse-phase negative mode HPLC-QTOF-MS to estimate the amount of diPT.