

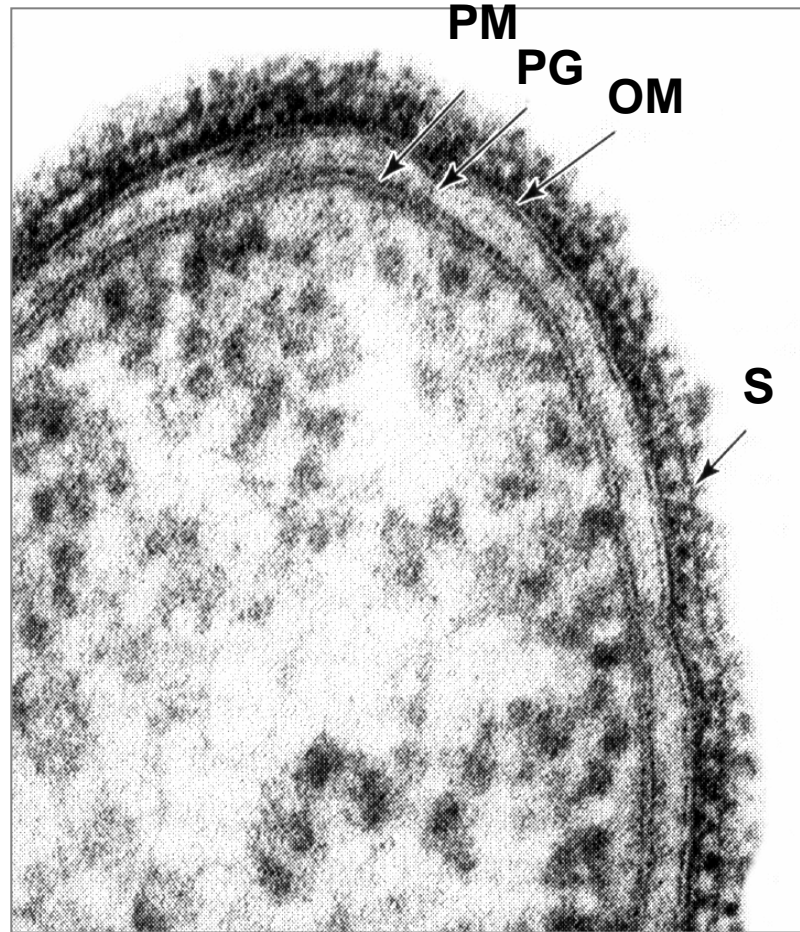
Structural Analysis of Bacterial Lipopolysaccharides – Past and Present

Otto Holst

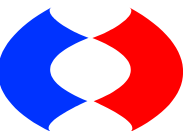
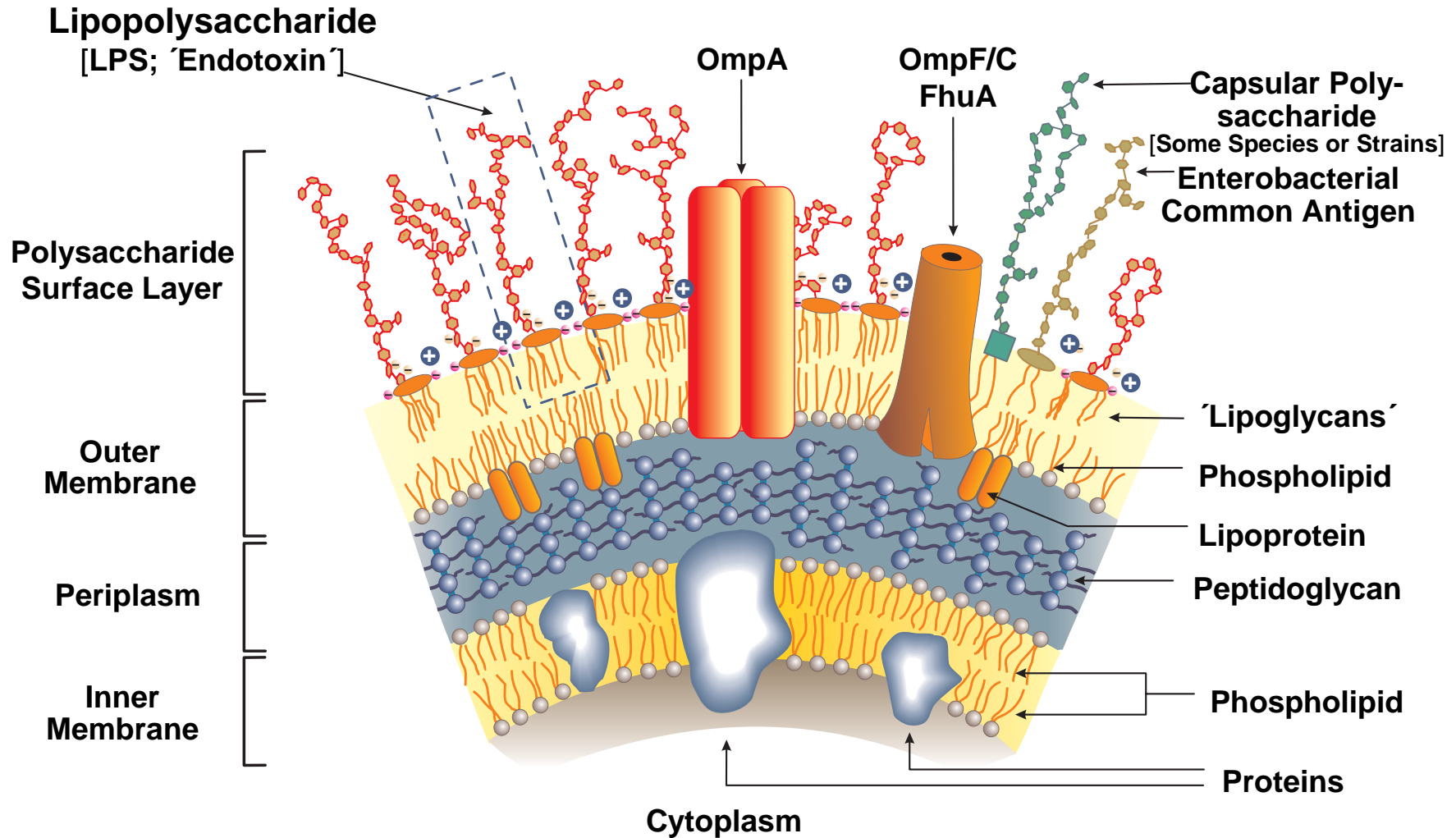
Research Center Borstel



The Gram-negative Cell Wall



Architecture of the Gram-negative Cell Wall

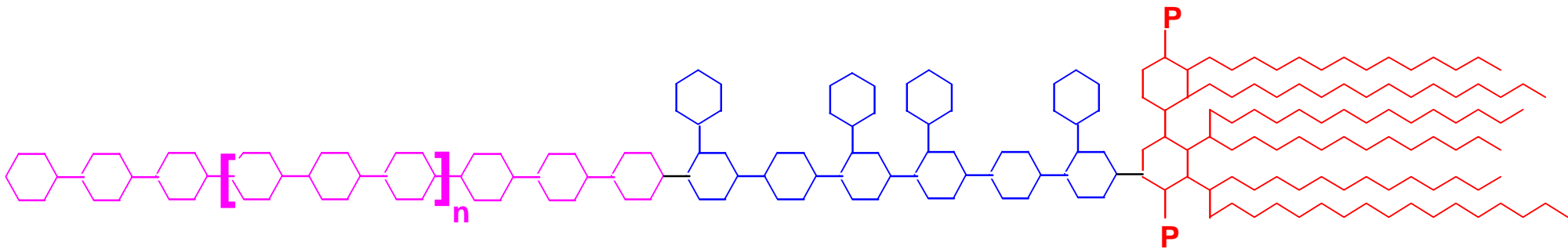


Schematic Structure of Lipopolysaccharides (LPS)

O-Antigen

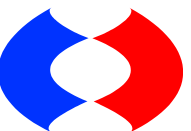
Core region

Lipid A

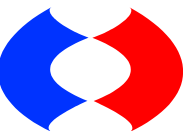
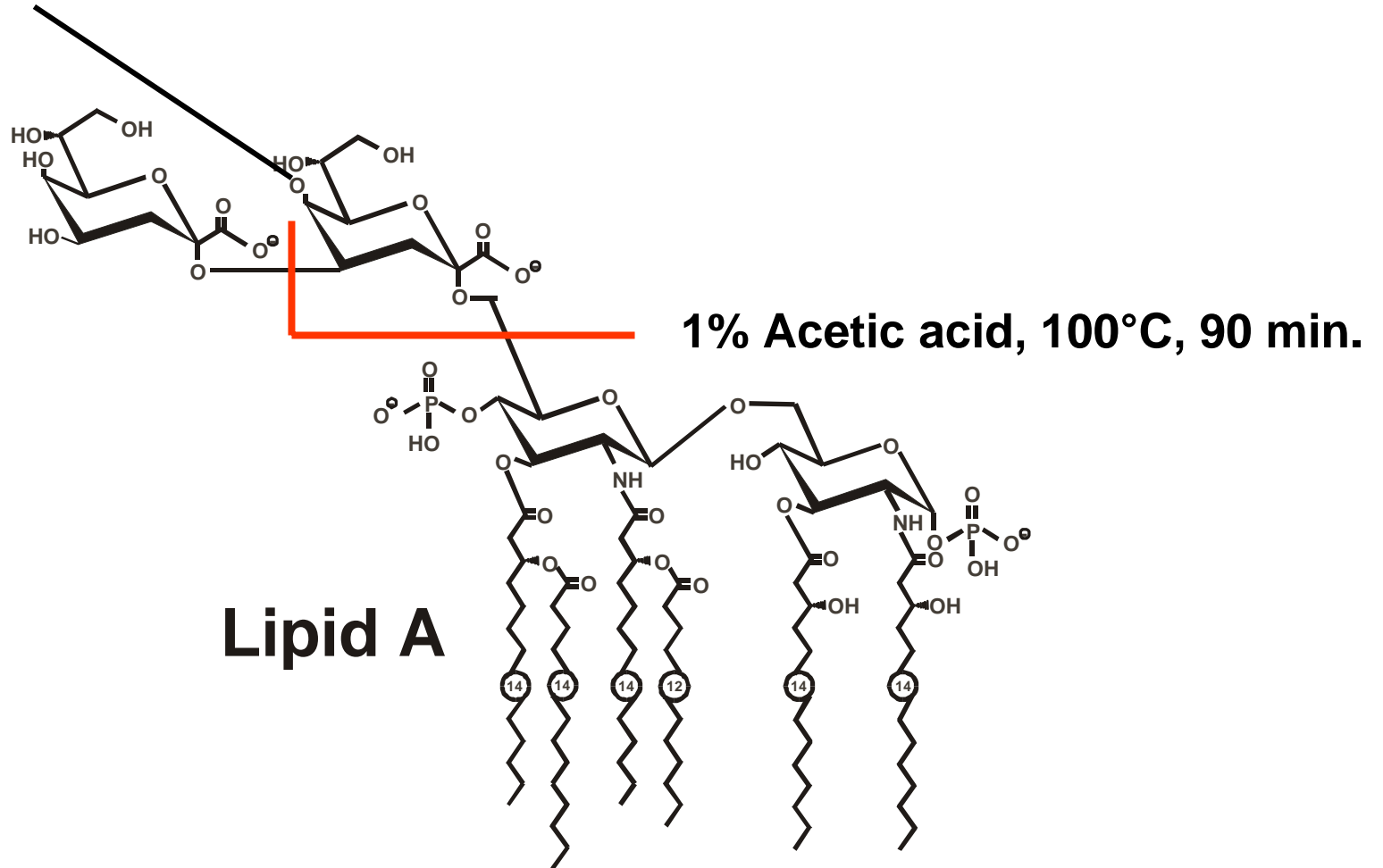


S-Form LPS

R-Form LPS



Core region





1. Ethanol
2. Aceton
3. Diethylether



Extraction:

**Hot Phenol/Water (S-form LPS) or
Phenol/Chloroform/Light Petroleum (R-Form LPS)**



Purification: Ultracentrifugation, RNase, DNase, Proteinase K

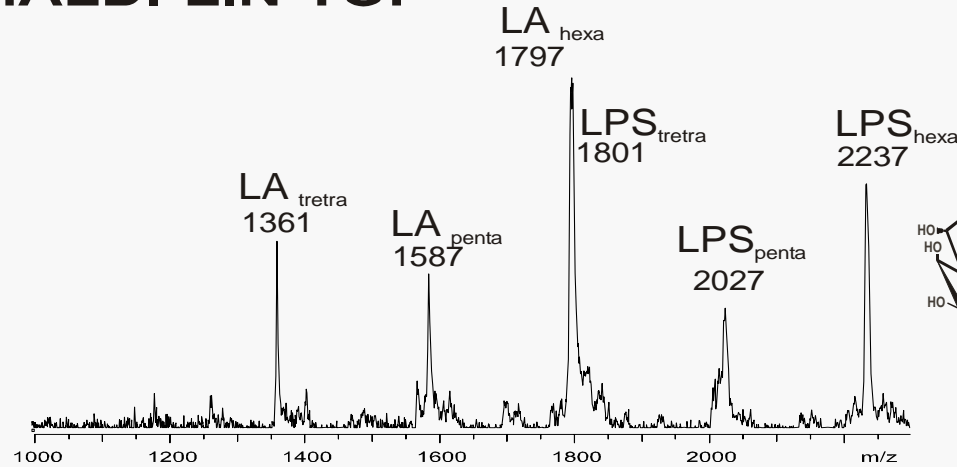
First Characterization: SDS-PAGE, MALDI-TOF- or FT-ICR-MS

**Compositional Analysis: Neutral and Amino Sugars, Uronic Acids,
Kdo, Phosphate, Fatty Acids**

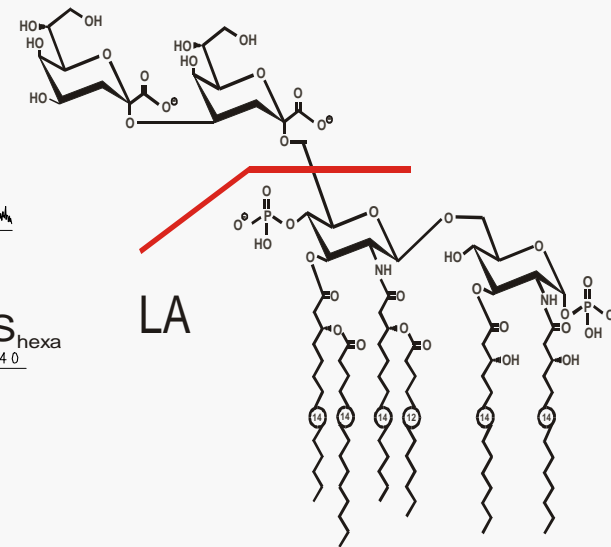


MS of *E.coli* Re-LPS (HL105)

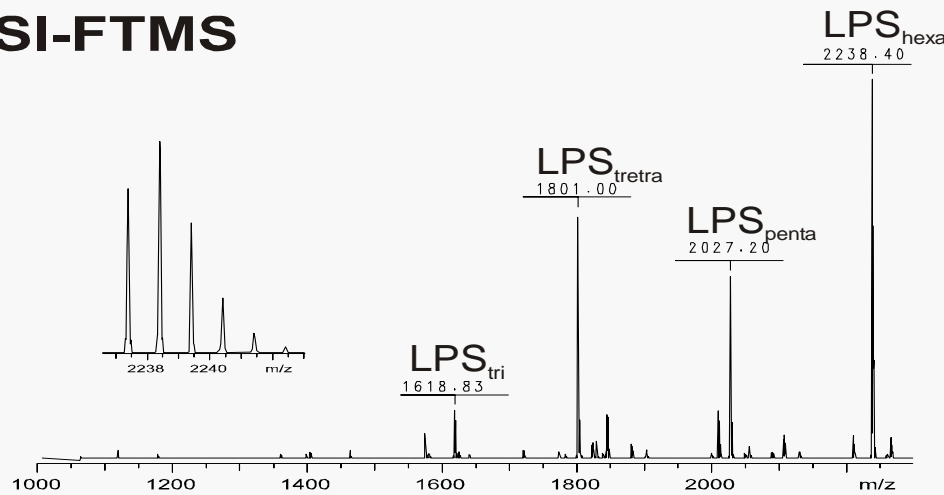
MALDI-LIN-TOF

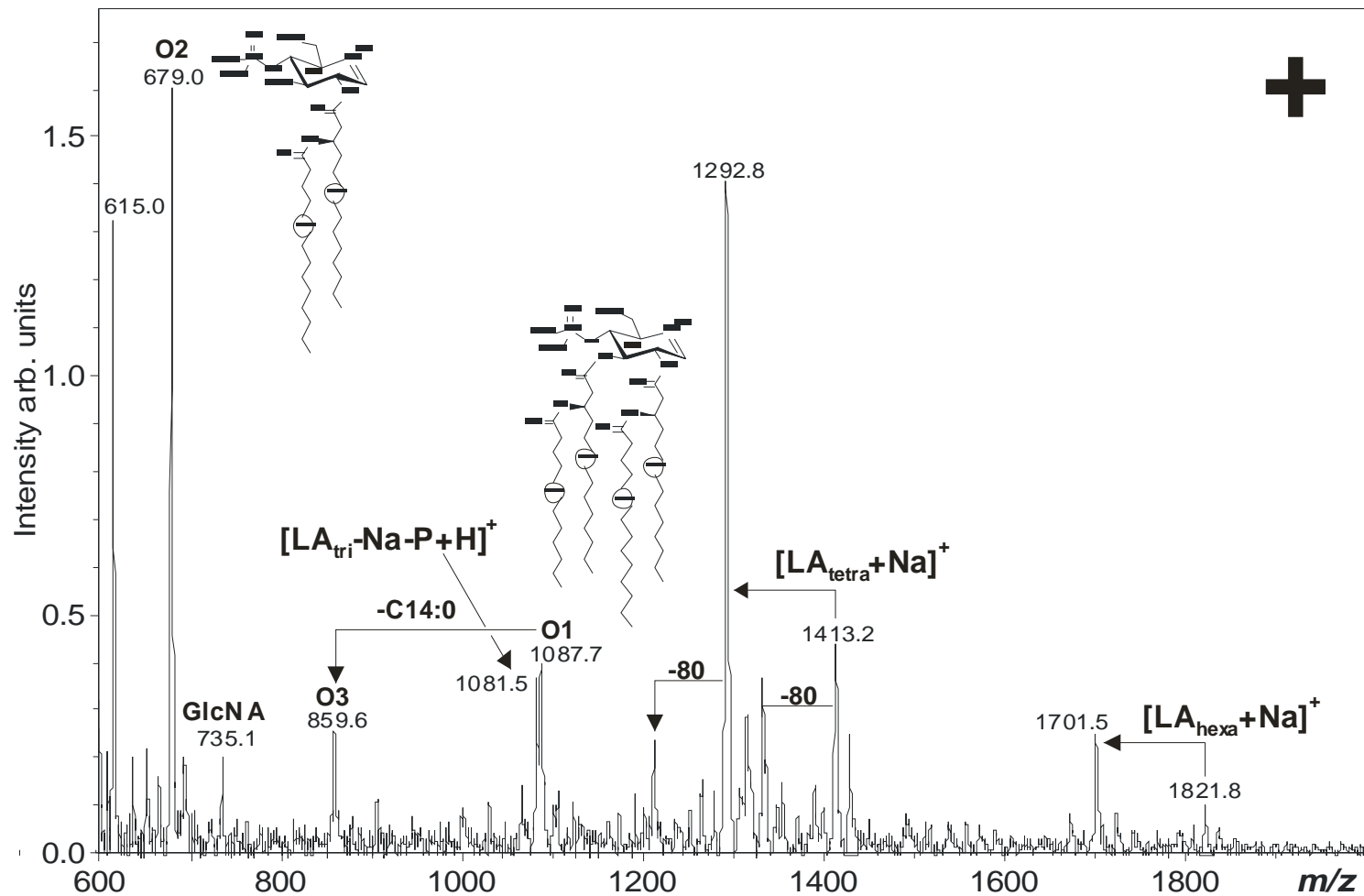


Courtesy B. Lindner

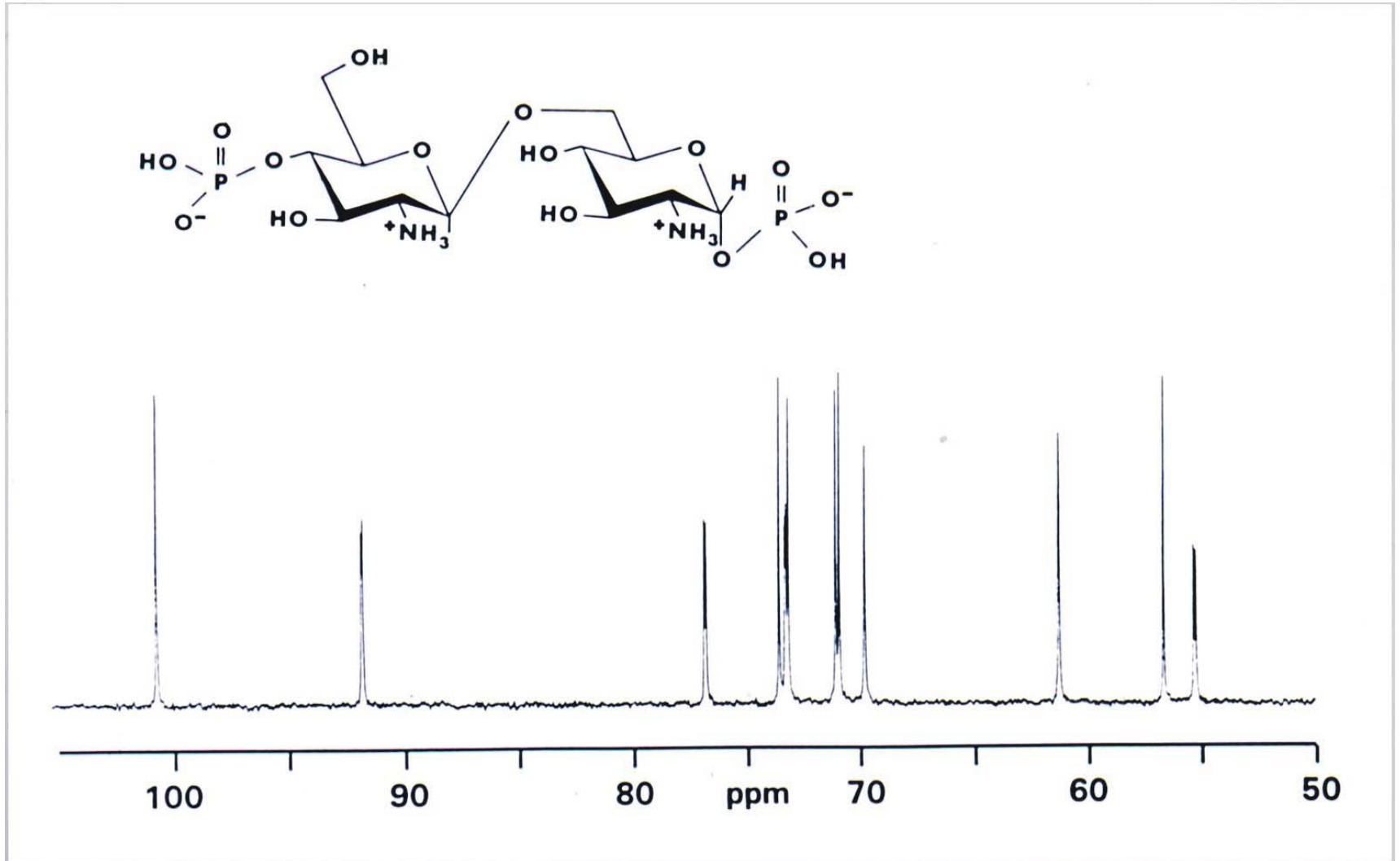


ESI-FTMS

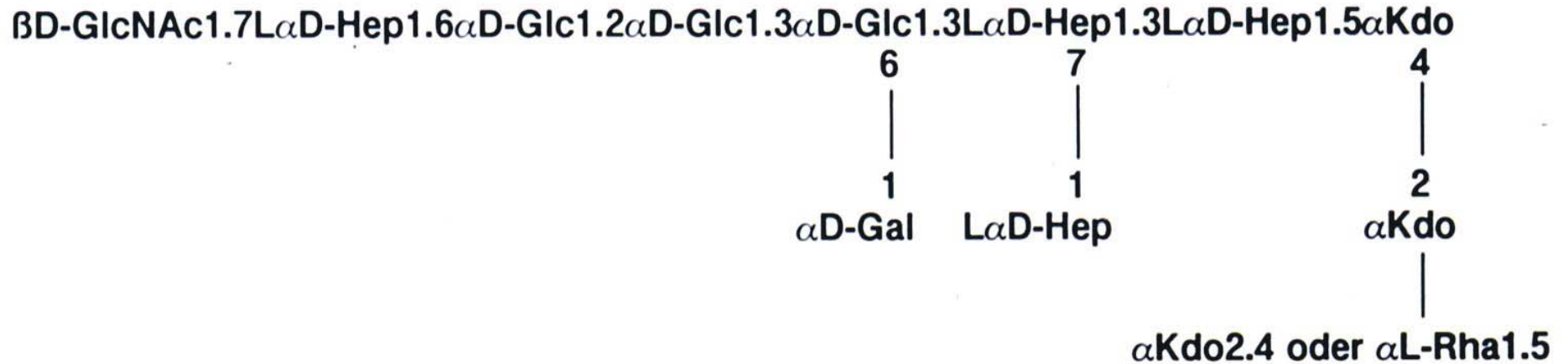




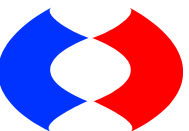
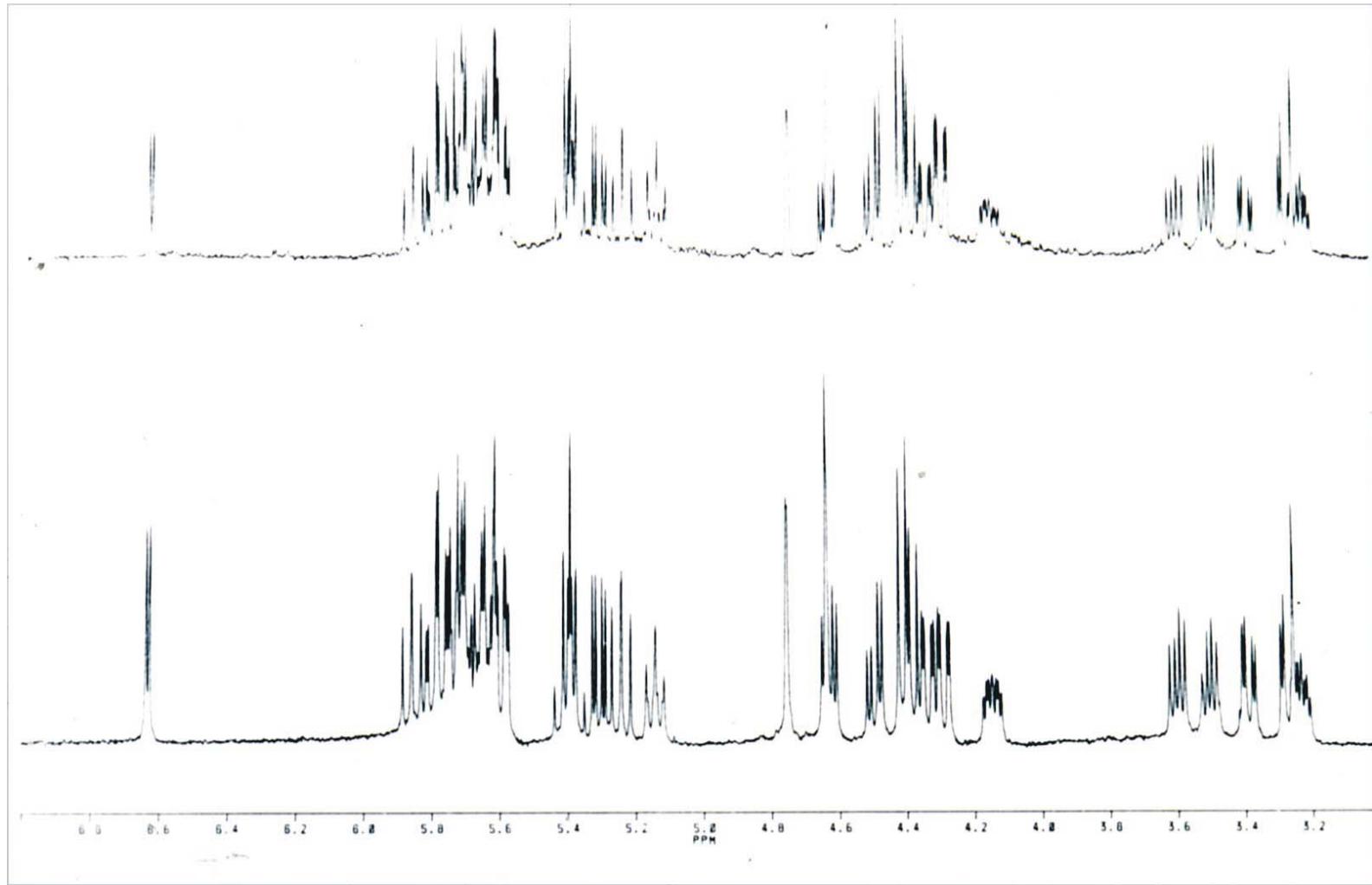
Lipid A \longrightarrow **Mild hydrazinolysis** \longrightarrow **4 M KOH, 120°C, 18 h**



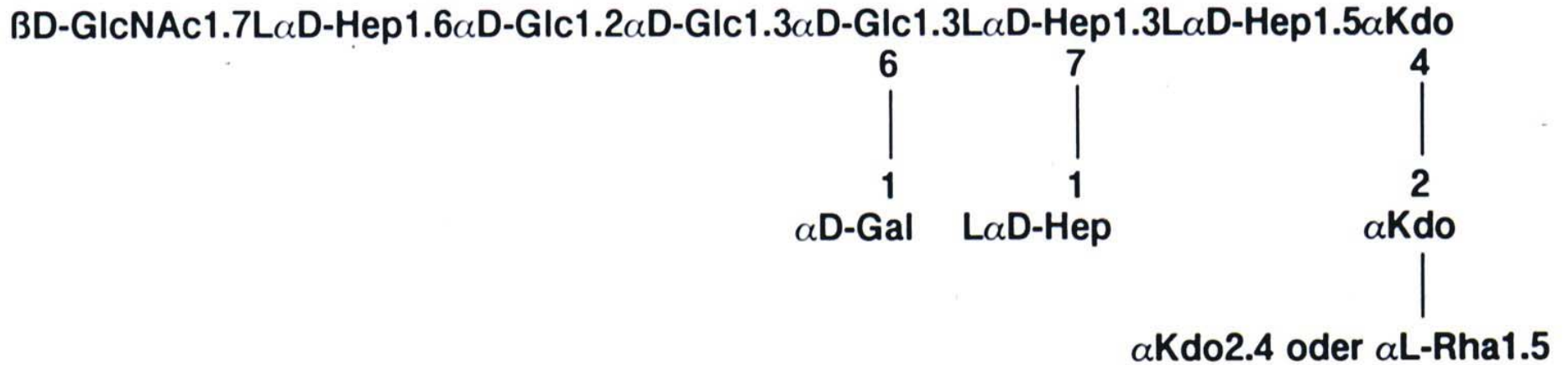
The Core Region of the LPS from *E. coli* K-12

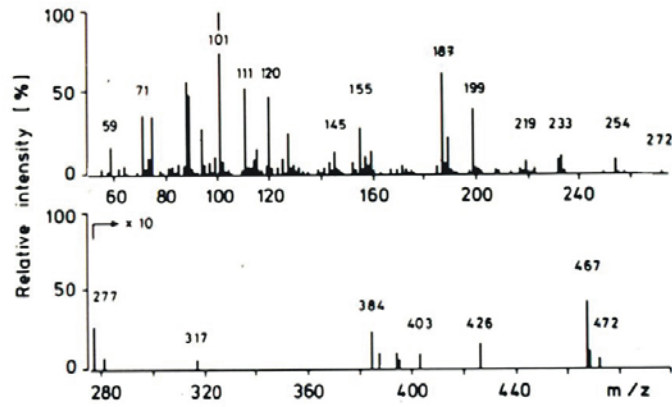
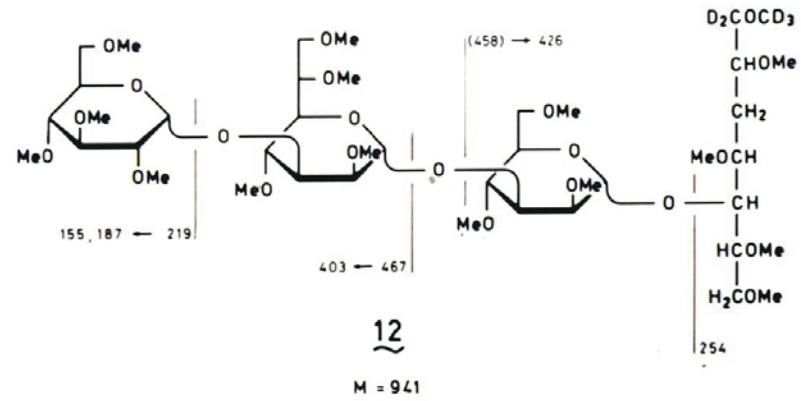


^1H NMR Spectra of Synthetic and Isolated Acetylated L- α -D-Hepp-(1-6)- α,β -D-Glcp



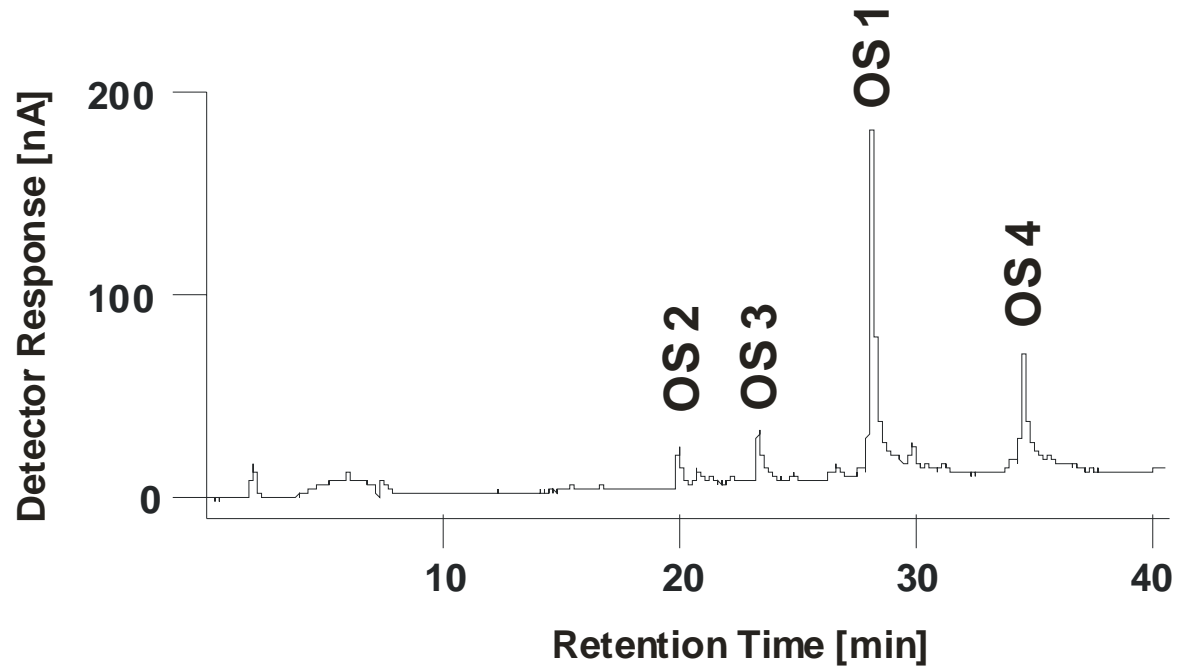
The Core Region of the LPS from *E. coli* K-12





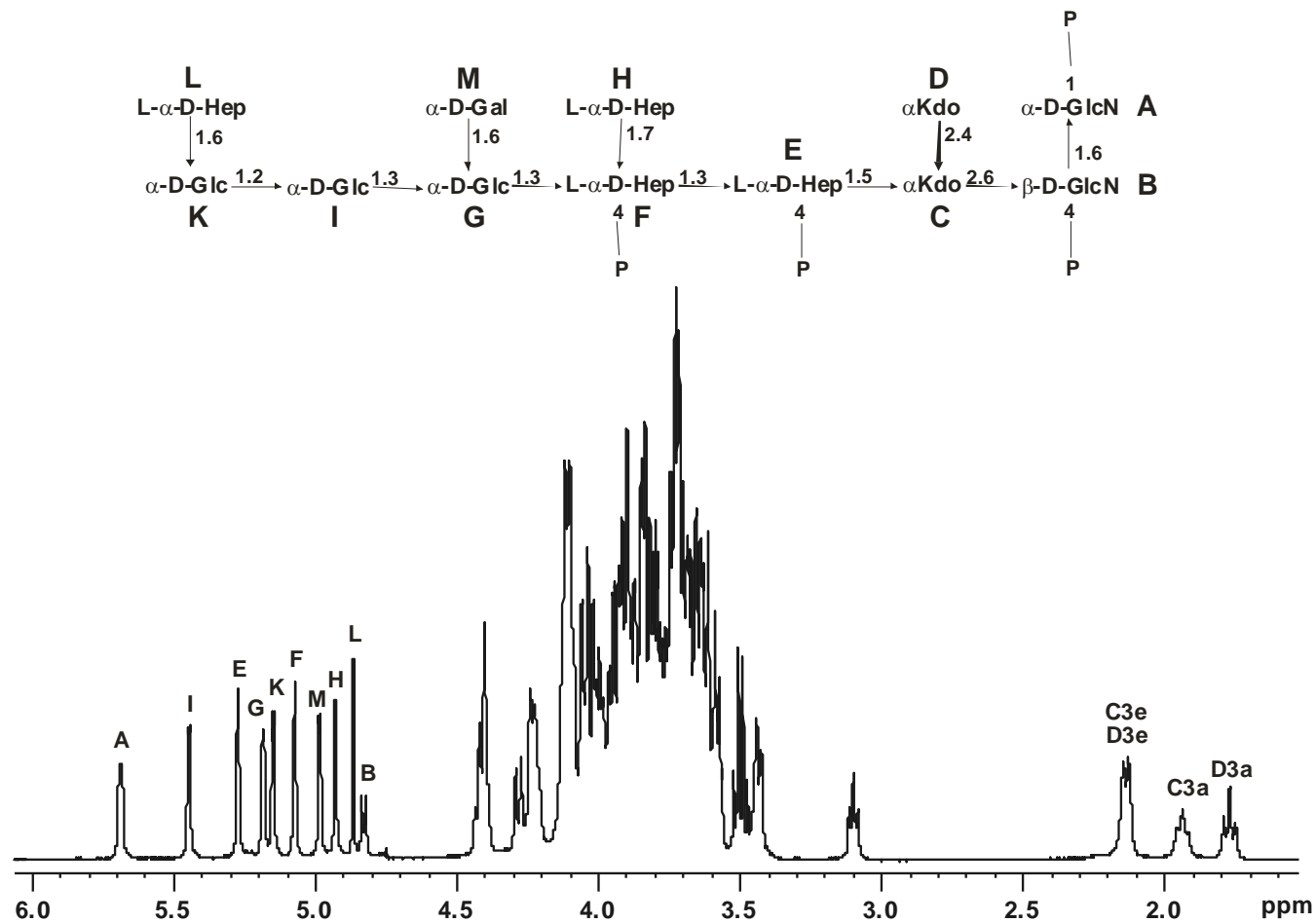
LPS → **Mild hydrazinolysis** → **4 M KOH, 120°C, 18 h**

HPAEC



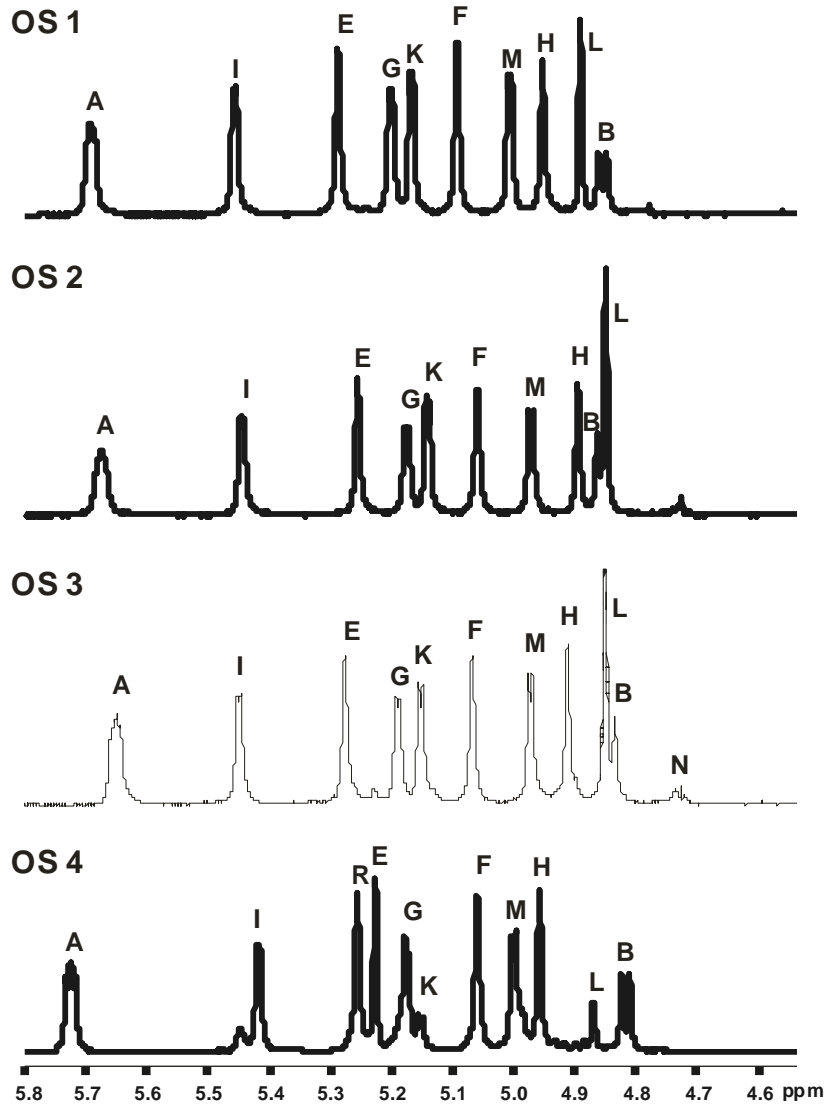
Courtesy S. Müller-Loennies





Courtesy S. Müller-Loennies

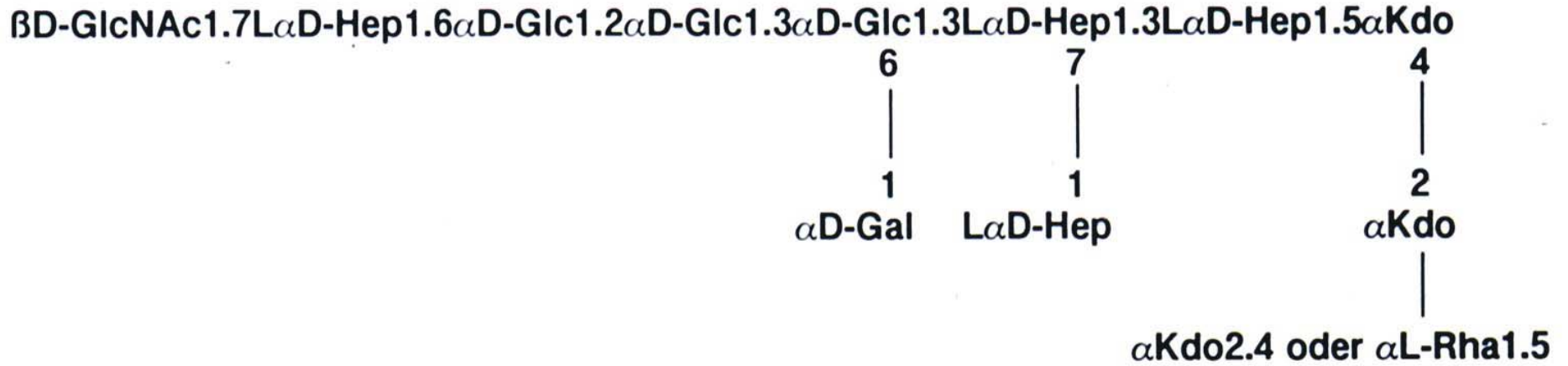




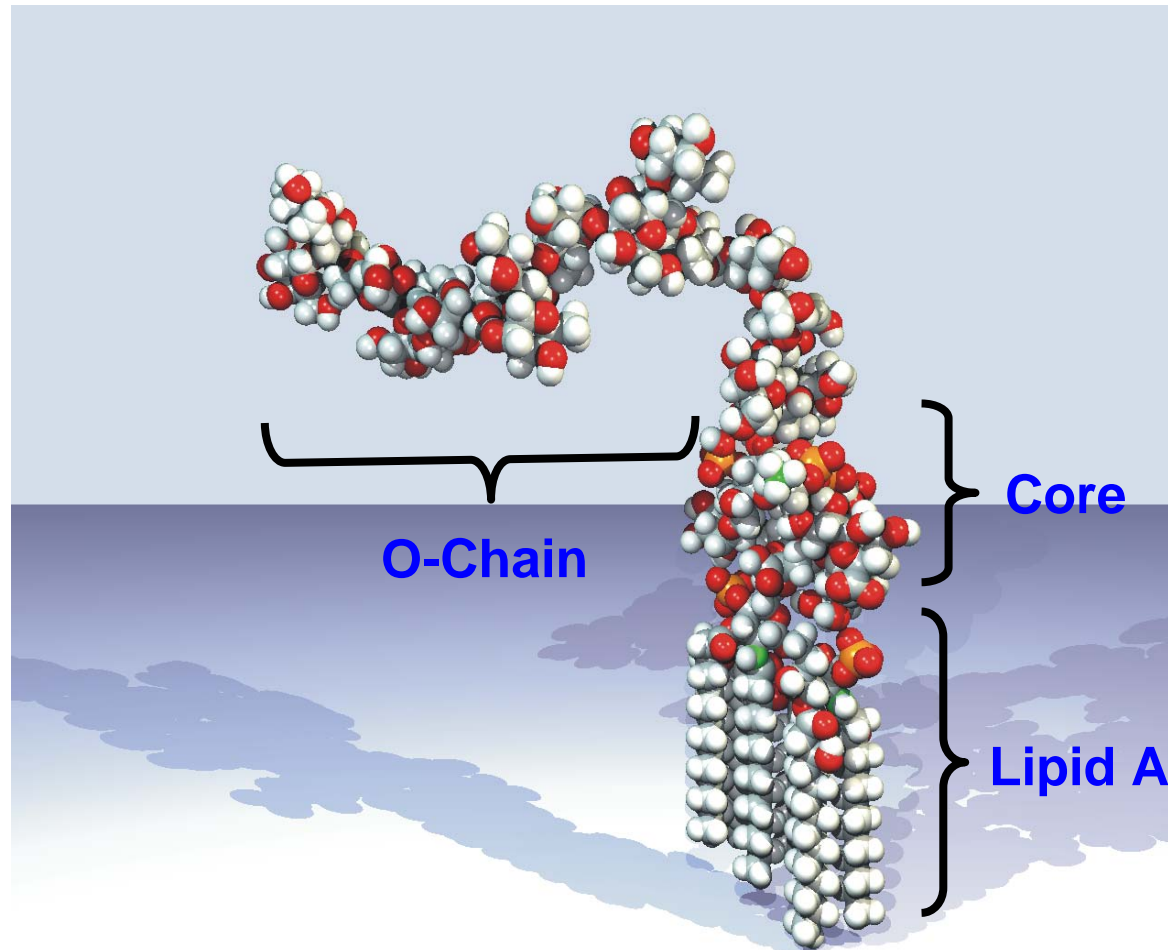
Courtesy S. Müller-Loennies



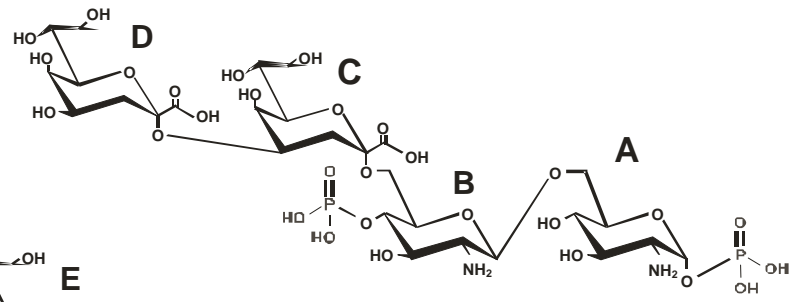
The Core Region of the LPS from *E. coli* K-12



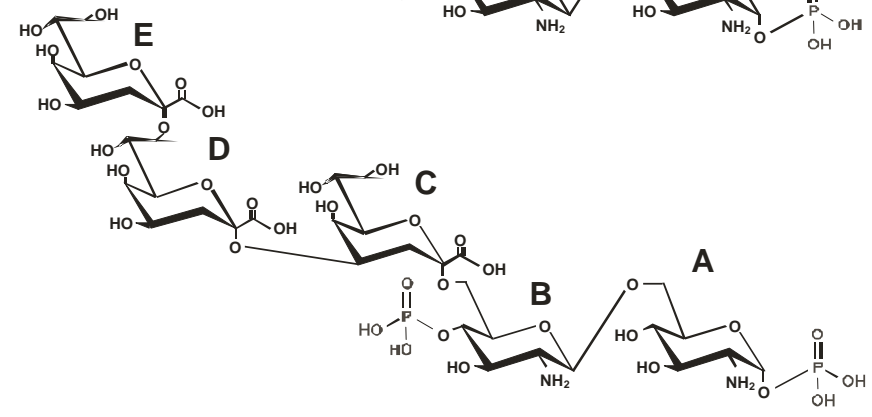
Lipopolysaccharide, LPS



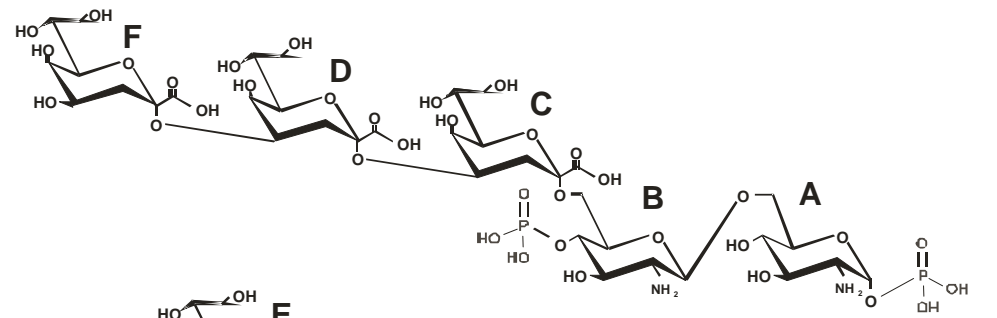
I



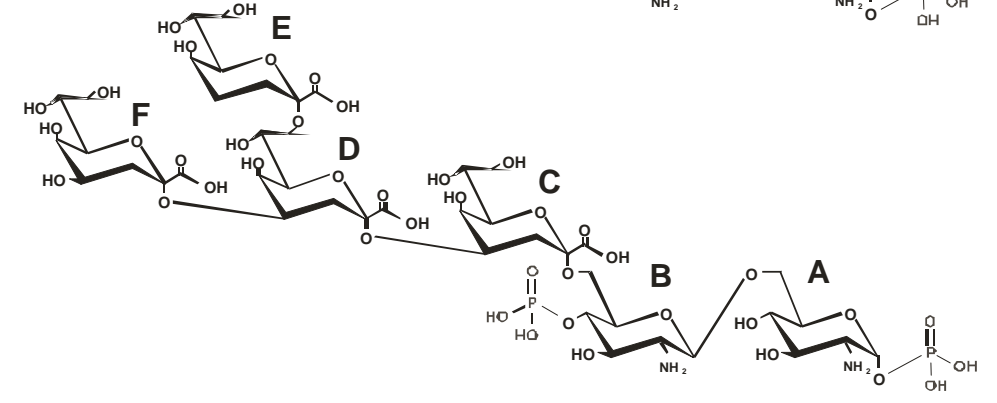
II

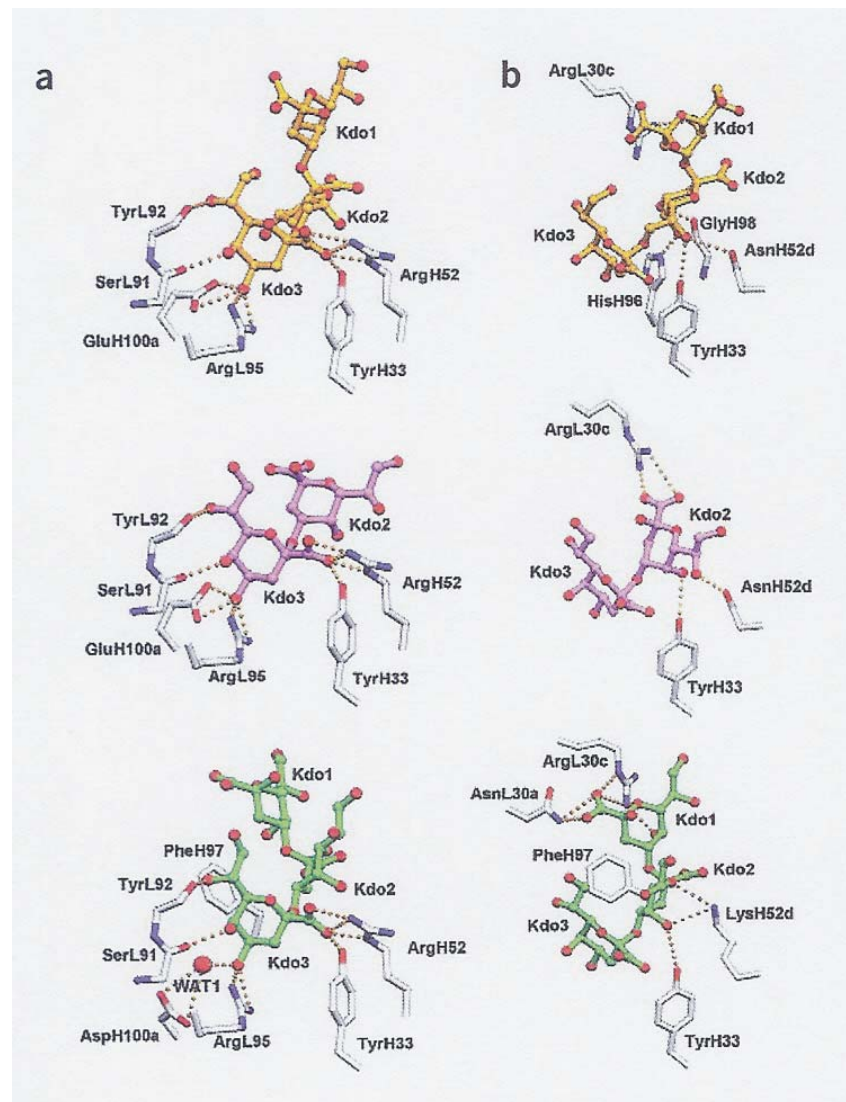


III



IV





Nguyen, Seto, MacKenzie, Brade, Kosma, Brade, Evans, *Nature Struct. Biol.* 10 (2003) 1019-1024



