

A new preparation of the saccharolipid glycan, Kdo_2 -Lipid A, is a nearly homogeneous Re lipopolysaccharide (LPS) substructure with endotoxin activity equal to that of native LPS. Kdo_2 -Lipid A is comparable to LPS and its activity is reduced by >10³ in cells from TLR-4 deficient mice. The advantage of Kdo_2 -Lipid A over LPS is that it is a reproducible, defined natural product, and it can be detected by ESI/MS at the low concentrations used to stimulate animal cells. The purity of Kdo_2 -Lipid A should also facilitate the structural analysis of its complexes with signaling receptors, such as TLR-4/MD2.



Raetz, C.R., Garrett, T.A., Reynolds, C.M., Shaw, W.A., Moore, J.D., Smith, D.C. Jr, Ribeiro, A.A., Murphy, R.C., Ulevitch, R.J., Fearns, C., Reichart, D., Glass, C.K., Benner, C., Subramaniam, S., Harkewicz, R., Bowers-Gentry, R.C., Buczynski, M.W., Cooper, J.A., Deems, R.A. and Dennis, E.A. (2006) Kdo₂-Lipid A of *Escherichia coli*, a defined endotoxin that activates macrophages via TLR-4. *J Lipid Res* 47, 1097-111.



Reference

Utilizing the uniform protocols established by the LIPID MAPS Consortium (www.lipidmaps.org), RAW 264.7 cells were treated with **Kdo₂-Lipid A**, a defined endotoxin that activates macrophages via TLR-4, then the sulfatide amounts and types were analyzed using liquid chromatography, electrospray tandem mass spectrometry. Sulfatides are detected and quantified by multiple reaction monitoring analysis in negative ion mode using an API 4000 quadrupole linear ion trap (varying the collision energy from 80 eV to 130 eV for the internal standard, a C12:0 homolog from Avanti Polar lipids, and the cellular sulfatides with C16:0 to 26:0 ceramide backbones). Although RAW 264.7 cells contain essentially no endogenous sulfatide, there is a large increase beginning approximately 12 h after Kdo₂-Lipid A addition. As best we have been able to ascertain, this is the first finding of induction of sulfatide biosynthesis by macrophage activation, and it will be interesting to determine if the increase in sulfatides has an impact on macrophage function. Quantitative analysis of sulfatides in RAW 264.7 cells treated with Kdo₂-Lipid A. (2007).

Jeremy Allegood, Elaine Wang, M. Cameron Sullards and Alfred H. Merrill, Jr. FASEB Journal. 21:779.12

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