ORGANIC LETTERS

2004
Vol. 6, No. 19
3333−3336

Preparation of a Lipid A Derivative That Contains a 27-Hydroxyoctacosanoic Acid Moiety

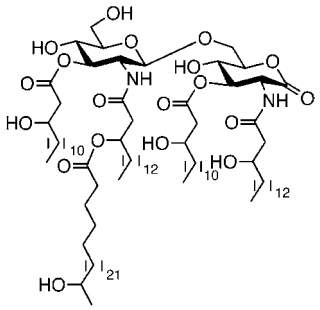
Balaji Santhanam and Geert-Jan Boons*

Complex Carbohydrate Research Center, The University of Georgia, 315 Riverbend Road, Athens, Georgia 30602

gjboons@ccrc.uga.edu

Received July 1, 2004

ABSTRACT



A general synthetic strategy for long-chain ω-1 hydroxy fatty acids has been developed, which employs as a key reaction step a cross metathesis between ω-unsaturated ester and 3-butene-2-ol. The resulting lipids were used for the preparation of lipid A derivatives of *Rhizobium sin-1*, which have the ability to inhibit the *E. coli* LPS-dependent synthesis of tumor necrosis factor by human monocytes.

Lipopolysaccharides (LPS), which are important components of the outer leaflet of the outer membrane of Gram-negative bacteria,[1] are major virulence factors of pathogenic bacteria[2,3] as well as for symbionts such as the nitrogen-fixing *Rhizobia* of legumes.[4] LPS consist of an O-chain polysaccharide, a core oligosaccharide, and an amphiphilic moiety referred to as lipid A. The structure of lipid A is largely conserved among most enteric bacteria, consisting of a β-(1−6)-linked glucosamine disaccharide backbone with phosphate monoesters at C-1 and C-4′ and β-hydroxyl fatty acyl groups and acyloxyacyl residues at positions 2 and 3, and 2′ and 3′, respectively (Figure 1). The nitrogen-fixing symbiont *Rhizobium sin-1* has a structurally unusual lipid A (Figure 1), differing in almost every aspect from those of enteric LPS.[5] Its disaccharide backbone is devoid of phosphate, and the

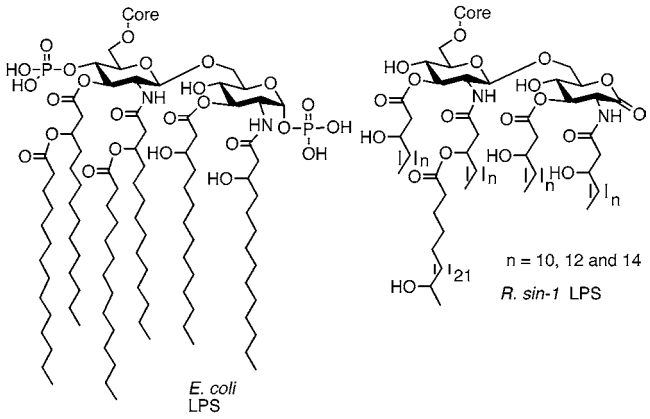


Figure 1. Structures of *E. coli* and *R. sin-1* LPS.

(1) Alexander, C.; Zahringer, U. *Trends Glycosci. Glycotechnol.* **2002**, *14*, 69−86.

(2) Rietschel, E. T.; Brade, H.; Holst, O.; Brade, L.; Muller-Loennies, S.; Mamat, U.; Zahringer, U.; Beckmann, F.; U., S.; Brandenburg, K.; Ulmer, A. J.; Mattern, T.; Heine, H.; Schletter, J.; Loppnow, H.; Schonbeck, U.; Flad, H. D.; Hauschildt, S.; Shade, F. U.; Di Padova, F.; Kusumoto, F.; Schumann, R. R. *Curr. Top. Microbiol. Immunol.* **1996**, *216*, 39−80.

(3) Raetz, C. R. H.; Whitfield, C. *Annu. Rev. Biochem.* **2002**, *71*, 635−700.

(4) Noel, K. D.; Vandenbosch, K. A.; Kulpaca, B. *J. Bacteriol.* **1986**, *168*, 1392−1401.

10.1021/ol048746f CCC: $27.50 © 2004 American Chemical Society
Published on Web 08/21/2004

glucosamine phosphate is replaced by 2-aminogluconolactone. Another unique structural feature is the presence of an unusual long-chain 27-hydroxyoctacosanoic acid (27OHC28:0) moiety, which in turn can be esterified by β-hydroxybutyrate. It has been suggested that the presence of 27OHC28:0 fatty acid in the lipid A of many Rhizobial species is required for maintaining the stability of the bacterial membrane during endocytotic invasion and is crucial for the survival of the bacterium within the plant-derived symbiosome compartment.[6−8] The 27OHC28:0 fatty acid is also present among a number of facultative intracellular pathogens that cause chronic infections such as *Brucella abortus*, *Bartonella henselae*, and *Legionella pneumophila*.[6]

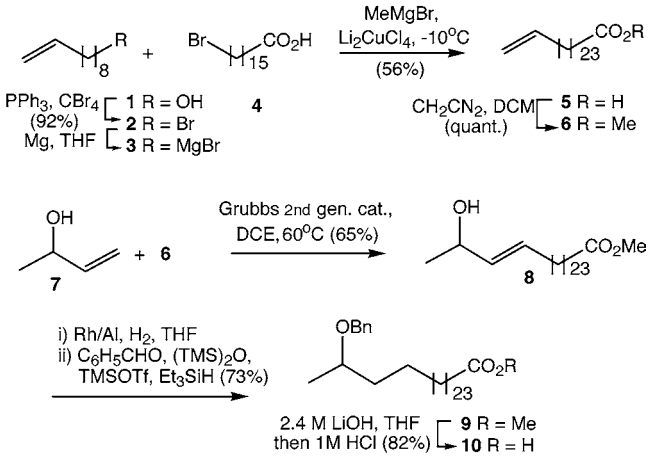
Recently, we demonstrated that LPS from *R. sin-1* inhibits the *E. coli* LPS-dependent synthesis of tumor necrosis factor (TNF-α) by human monocytes.[9,10] An LPS-mediated overproduction of host-derived inflammatory mediators such as TNF-α may result in septicemia, which is a life-threatening syndrome for which currently no treatment exists but supportive therapy in an intensive care unit setting.[11,12] Thus, compounds such as *R. sin-1* LPS may have the potential to prevent the deleterious effects of enteric LPS. As a result of the inherent molecular heterogeneity of *R. sin-1* LPS, it cannot be developed as a therapeutic agent for Gram-negative sepsis. We have, however, demonstrated[10] that a synthetic analogue of the lipid A of *R. sin-1* emulates the ability of heterogeneous *R. sin-1* LPS to antagonize enteric LPS, albeit with somewhat higher IC_{50} values. The synthetic compound contained an octacosanoic acid moiety rather than the natural hydroxylated 27-hydroxyoctacosanoic acid.

To determine the contributions of the hydroxylation of the long-chain 27-hydroxyoctacosanoic acid moiety for antagonistic properties, an efficient preparation of this fatty acid was required. Furthermore, it was necessary that a synthetic procedure was developed that allowed the 27-hydroxyoctacosanoic acid moiety to be introduced at a late stage of synthesis. The new synthetic approach would also need to allow an easy incorporation of other fatty acids for structure−activity relationship studies.

An efficient approach for the chemical synthesis of long-chain ω-1 hydroxy fatty acids (>C18) has not yet been reported. These compounds have been obtained by an enzymatic hydroxylation of inexpensive saturated fatty acid,[13] a procedure that gave, however, mixtures of compounds in which ω-2 and ω-3 were also hydroxylated.

We envisaged that olefin cross metathesis between an ω-unsaturated ester (e.g., **6**, Scheme 1) and 3-butene-2-ol

Scheme 1. Preparation of 27-Hydroxy-octacosanoic Acid

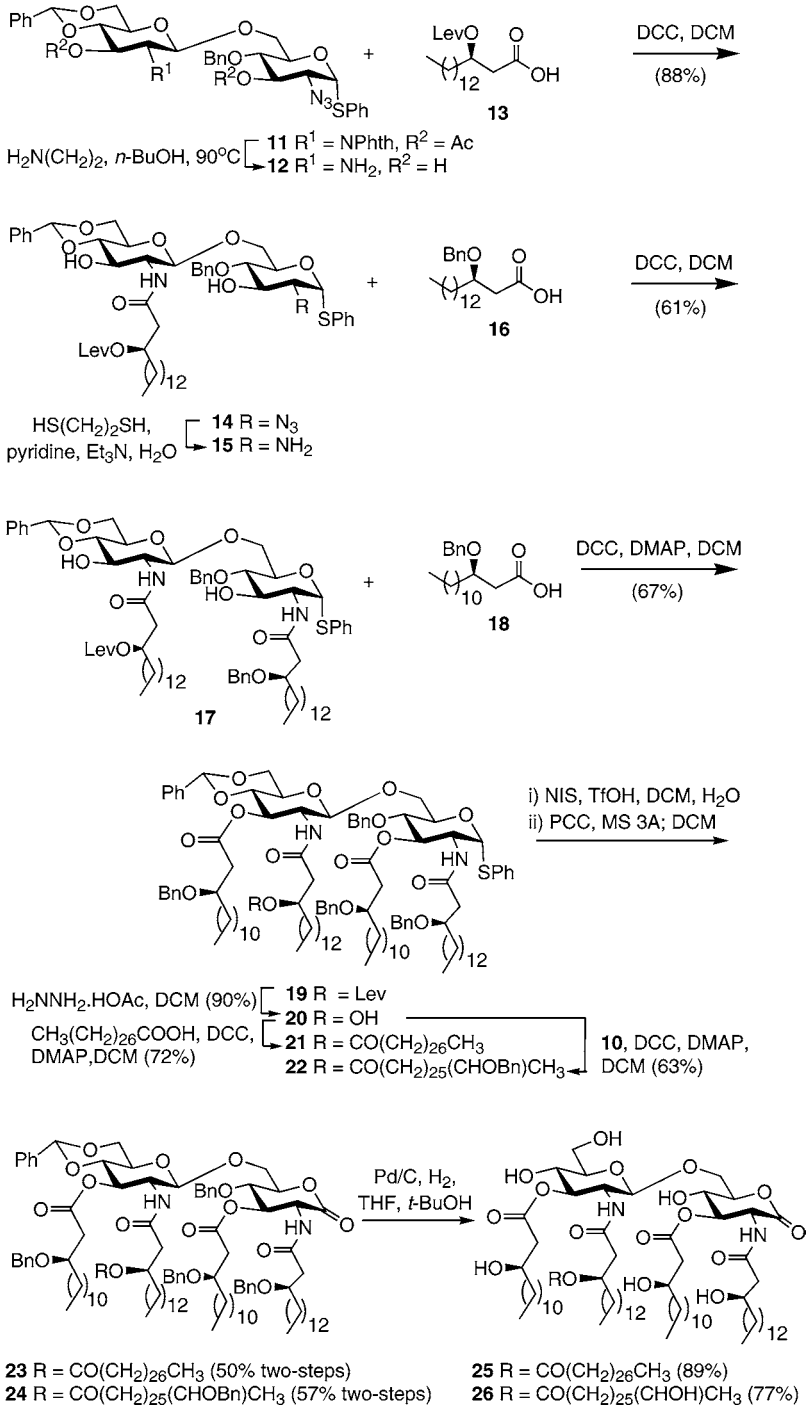


(**7**) would give access to any ω-1 hydroxyl fatty ester after the reduction of the double bond. In general, high selectivity in olefin cross metathesis can be achieved when the two olefins have significantly different reactivities. In this respect, it has been shown that secondary allylic alcohols are of lower reactivity than terminal olefins.[14] Thus, it was expected that a metathesis reaction of **6** with **7** would give a product in good overall yield.

Methyl 25-hexacosenoiate (**6**), which is a starting material for the cross metathesis reaction, was prepared from commercially available bifunctional starting materials of appropriate chain lengths (Scheme 1). Thus, reaction of 9-decen-1-ol (**1**) with CBr_4 and PPh_3 gave bromide **2** in a yield of 92%, which was converted into a cuprate by reaction with magnesium followed by transmetalation with dilithium tetrachlorocuprate (Li_2CuCl_4). Condensation of the cuprate with bromide **4** gave 25-hexacosenoic acid (**5**),[15] which was transformed into the corresponding methyl ester **6** by treatment with freshly prepared diazomethane. A cross metathesis reaction[14] of ω-unsaturated ester **6** and 3-butene-2-ol (**7**) using Grubbs first generation catalyst gave **8** in a low yield of 15%. Fortunately, when Grubbs second generation catalyst was employed, compound **8** was isolated in a much improved yield of 65% as mainly the *trans*-isomer (*E*/*Z* = 20:1). A significantly lower yield of the cross metathesis product was obtained when the hydroxyl of **7** was protected as an acetyl ester or benzyl ether. Next, the double bond of **7** was reduced by hydrogenation over Rh/alumina to give **8**. Finally, benzylation of the ω-1 hydroxyl of **8** by treatment with benzaldehyde, TMS_2O, TMSOTf, and Et_3SiH (→ **9**) followed by saponification of the methyl ester with LiOH gave **10** in a 73% overall yield (two steps).

(5) Jeyaretnam, B.; Glushka, J.; Kolli, V. S. K.; Carlson, R. W. *J. Biol. Chem.* **2002**, *277*, 41802−41810.
(6) Bhat, U. R.; Carlson, R. W.; Busch, M.; Mayer, H. *Int. J. Syst. Bacteriol.* **1991**, *41*, 213−217.
(7) Kannenberg, E. L.; Carlson, R. W. *Mol. Microbiol.* **2001**, *39*, 379−391.
(8) Vedam, V.; Kannenberg, E. L.; Haynes, J. G.; Sherrier, D. J.; Datta, A.; Carlson, R. W. *J. Bacteriol.* **2003**, *185*, 1841−1850.
(9) Van den Plas, M. L.; Carlson, R. W.; Jeyaretnam, B.; McNeill, B.; Barton, M. H.; Norton, N.; Murray, T. F.; Moore, J. N. *J. Biol. Chem.* **2002**, *277*, 41811−41816.
(10) Demchenko, A. V.; Wolfert, M. A.; Santhanam, B.; Moore, J. N.; Boons, G. J. *J. Am. Chem. Soc.* **2003**, *125*, 6103−6112.
(11) Roberts, F. J.; Geere, I. W.; Coldman, A. *Rev. Infect. Dis.* **1991**, *13*, 34−46.
(12) Pittet, D.; Tarara, D.; Wenzel, R. P. *J. Am. Med. Assoc.* **1994**, *271*, 1598−1601.
(13) Schneider, S.; Wubbolts, M. G.; Sanglard, D.; Witholt, B. *Tetrahedron: Asymmetry* **1998**, *9*, 2833−2844.
(14) Chatterjee, A. K.; Choi, T. L.; Sanders, D. P.; Grubbs, R. H. *J. Am. Chem. Soc.* **2003**, *125*, 11360−11370.
(15) Mirviss, S. B. *J. Org. Chem.* **1989**, *54*, 1948−1951.

Scheme 2. Synthesis of *R. sin-1* Lipid A



Having successfully prepared 27-benzyloxy-octacosanoic acid **10**, attention was focused on the preparation of lipid A derivative **26** (Scheme 2), which contains this fatty acid moiety. Lipid A derivatives are usually prepared by first synthesizing β-hydroxy acyl- and acyloxoacyl acids, which are then condensed with amines or alcohols of an appropriately protected saccharide. We envisaged that an acylation of the β-hydroxyl of the C-2′ myristate moiety of **20** with 27-hydroxyoctacosanoic acid **10** would offer a more convergent approach and efficient use of expensive starting material. Furthermore, the hydroxyl of this advanced intermediate can be acylated with other fatty acids (e.g., octacosanic acid leading to compound **25**), thus creating an opportunity for the convenient preparation of a library of compounds for structure−activity relationship studies.

Compound **20** could easily be prepared from the selectively protected disaccharide **11**[10] and Lev ester or benzyl ether protected β-hydroxy fatty acids **13**,[16] **16**, and **18**[17]

(16) Duynstee, H. I.; van Vliet, M. J.; van der Marel, G. A.; van Boom, J. H. *Eur. J. Org. Chem.* **1998**, 303−307.

(17) Oikawa, M.; Kusumoto, S. *Tetrahedron: Asymmetry* **1995**, *6*, 961−966.

(Scheme 2). Thus, removal of the phthalimido group and acetyl esters of **11** by treatment with ethylenediamine in refluxing *n*-butyl alcohol followed by selective *N*-acetylation of **12** with **13** in the presence of DCC gave **14** in an overall yield of 88%. Reduction of the azido moiety of **14** was easily accomplished by reaction with 1,3-propanedithiol in a mixture of pyridine, triethylamine, and water,[18] and the amine of the resulting compound **15** was acylated with **16** using DCC as the activating reagent to give **17** in an overall yield of 55%. The C-3 and C-3′ hydroxyls of **17** were acetylated with **18** using DCC and DMAP to give **19** in 67% yield. Thus, by employing the DDC-mediated acylation in the presence or absence of DMAP, selectivity between O- and N-acylation could be accomplished.

Selective removal of the Lev ester of **19** could easily be achieved by treatment with hydrazine acetate[19] to give the advanced intermediate **20** in an excellent yield. The hydroxyl of **20** was then acylated with octacosanic acid or 27-benzyloxy-octacosanoic acid **10** using DCC and DMAP as the activating reagent to give **21** and **22**, respectively. Hydrolysis of the thiophenyl moiety of **21** and **22** was accomplished with NIS in wet dichloromethane in the presence of TfOH.[20] The corresponding lactols (α/β mixtures) were then oxidized with PCC to the lactones **23** and **24**, respectively. Finally, the benzyl ethers and benzylidene acetal of **23** and **24** were removed by catalytic hydrogenation over Pd/C to afford the target compounds **25** and **26**, respectively.

In conclusion, an efficient synthetic approach for ω-unsaturated ester has been developed by olefin cross metathesis between an ω-unsaturated ester and 3-butene-2-ol followed by a reduction of the double bond of the resulting product. Furthermore, it has been demonstrated that an acylation of the β-hydroxyl of the myristate moiety of a lipid A precursor (e.g., **20**) gives easy access to a range of compounds for structure−activity relationship studies. In this study we have employed two different fatty acids, namely, the octacosanoic acid and 27-hydroxyoctacosanoic acid, to clarify the effect of the ω-1 hydroxyl group to antagonistic properties of natural *Rhizobial* lipid A. The results of these biological studies will be reported elsewhere.

Acknowledgment. This research was supported by the NIH Research Resource Center for Biomedical Complex Carbohydrates (P41-RR-5351).

Supporting Information Available: Experimental procedures and ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

OL048746F

(18) Bayley, H.; Standring, D. N.; Knowles, J. R. *Tetrahedron Lett.* **1978**, 3633−3634.

(19) Van Boom, J. H.; Burgers, P. M. J. *Tetrahedron Lett.* **1976**, 4875−4878.

(20) Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331−1334.