Total Synthesis of the Proposed Structure of *Rhodobacter sphaeroides* Lipid A Resulting in the Synthesis of New Potent Lipopolysaccharide Antagonists

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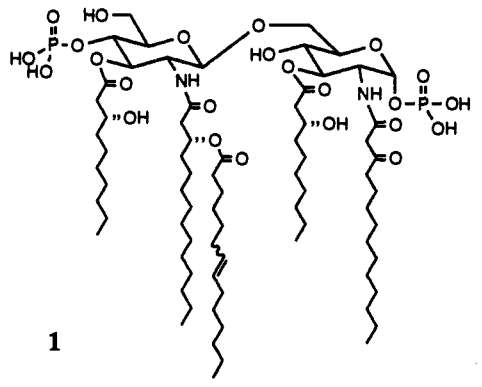
Lipopolysaccharide (LPS) or endotoxin is known to elicit a variety of pathological effects as a result of an adverse host inflammatory response.[1] The terminal disaccharide phospholipid subunit of LPS, known generically as lipid A, is responsible for triggering these events.[2] Recently, nontoxic lipid A's were isolated from bacterial extracts and shown to exhibit LPS antagonistic properties.[3] These properties are useful for investigations into the mechanisms of LPS action and have served as the basis for speculation about strategies for the possible therapeutic intervention in LPS-related disease states. It is tempting to develop structure–activity relationships among lipid A analogs as an aid toward understanding LPS action at the molecular level. However, such an approach critically depends upon firm structural information and supplies of homogeneous materials. Qureshi and co-workers[3] have isolated a potent LPS antagonist from *Rhodobacter sphaeroides* (abbreviated as Rs-DPLA) as an inseparable mixture of three compounds. On the basis of degradation and spectral studies, they have suggested the structure **1** for the major lipid A component. In this communication we



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report (1) the first total synthesis of the proposed Rs-DPLA structure, (2) comparison of the synthetic material with the natural Rs-DPLA, disproving the proposed structure, and (3) remarkable biological activity observed for the synthetic lipid A's **1a** and **1b**.

Existing lipid A synthetic methodologies rely on hydrogenolysis to remove the benzyl protecting groups at the final stage of synthesis.[4] The 1,3-ketoamido and olefinic functionalites present in the proposed Rs-DPLA are incompatible with these reaction conditions. Our synthetic strategy was formulated around the use of the allyl carbonate (AOC) protecting group, which could be removed at the final step without affecting these functionalites.

The readily available azido sugar **2**[5] was selected as a starting material because it could easily be transformed into both glycosyl donor **4** and acceptor **5** via the common intermediate **3**, as shown in Scheme 1. Key intermediate **3** was prepared in two steps from **2** and (*R*)-3-(((allyloxy)carbonyl)oxy)decanoic acid[6] in 80% overall yield. Treatment of **3** with pyridine and allyl chloroformate selectively gave the 6-monoprotected intermediate **4** in 77% yield. Phosphorylation (via the two-step phosphitylation/phosphorlation procedure[9]) at the sterically crowded 4-position was easily achieved without 4,6-carbonate formation by bis(allyloxy)-(diisopropylamino)phosphine[9] under mild acidic conditions in 80% yield. Removal of the protecting group at the anomeric position with HF, followed by treatment in neat CCl_3CN in the presence of K_2CO_3, afforded the desired glycosyl donor **4** in 69% yield as an anomeric mixture of trichloroacetimidates.

Glycosyl acceptor **5** was efficiently synthesized from **3** by a five-step sequence in 70% overall yield (Scheme 1). First, the primary alcohol of **3** was protected using *tert*-butyldimethylsilyl chloride in 90% yield. An AOC group was incorporated at the 4-position by the sequential addition of phosgene followed by allyl alcohol in 80% yield. Rapid and clean reduction of the azido group (less than 15 min) at the 2-position by tin(II) tris-(benzenethiolate)–triethylamine complex,[10] followed by condensation of the resulting amine with 3-oxotetradecanoic acid,[11] gave the desired ketoamide intermediate in 70% overall yield. Use of HF cleanly and selectively removed the 6-position TBS to give **5** in 80% yield.

Glycosyl acceptor **5** and donor **4** were then coupled in the presence of silver triflate[12] in hexanes to give a 1:2 ratio of α- and β-disaccharides **6** (Scheme 2). The azido group of the isolated β-disaccharide **6** was cleanly reduced by tin(II) tris(benzenethiolate)–triethylamine complex to give amine **7** in 80% yield. Since the configuration of the Δ^7 olefin had not been determined,[3] acylation of amine **7** with both *cis*- and *trans*-Δ^7-tetradecenoyl-(*R*)-3-oxotetradecanoates[13,15] was conducted to give **8a** and **8b**, respectively, in 70% overall yield from **6**.

Removal of the protecting group at the anomeric position of **8a** and **8b** with HF, followed by phosphorylation with bis(allyloxy)-(diisopropylamino)phosphine, provided exclusively the α-phosphates **9a** and **9b**, respectively, in 80% isolated yield (Scheme 3). Final deprotection was accomplished by treatment of **9a** and **9b** with $(Ph_3P)_4Pd$,[17] followed by purification on DEAE cellulose,[18]

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(2) Homma, J.; Kanegasaki, S.; Luderitz, O.; Shiba, T.; Westphal, O. *Bacterial Endotoxin: Chemical, Biological and Clinical Aspects* ; Verlag Chemie: Basel, 1984.
(3) (a) Qureshi, N.; Honovich, J. P.; Hara, H.; Cotter, R. J.; Takayama, K. *J. Biol. Chem.* **1988**, *263*, 5502–5504. (b) Qureshi, N.; Takayama, K.; Kurtz, R. *Infect. Immunol.* **1991**, *59*, 441–444.
(4) Imoto, M.; Yoshimura, H.; Shimamoto, T.; Sakaguchi, N.; Kusumoto, S.; Shiba, T. *Tetrahedron Lett.* **1985**, *26*, 1545–1548.
(5) Schmidt, R.; Kinzy, W. *Tetrahedron Lett.* **1987**, *28*, 1981–1984.
(6) (*R*)-3-(((Allyloxy)carbonyl)oxy)decanoic acid was synthesized in six steps starting from *n*-heptyl cyanide: (i) methyl bromoacetate/Zn dust/THF/reflux then 1.0 N HCl;[7] (ii) H_2 (1500 psi)/Noyori catalyst[8]/THF/rt; (iii) NaOH 2.5 M/rt/THF; (iv) 2-bromoacetophenone/EtOAc/TEA/rt; (v) phosgene/toluene/0 °C followed by allyl alcohol; (vi) Zn dust/AcOH/rt.
(7) Hannick, S.; Kishi, Y. *J. Org. Chem.* **1983**, *48*, 3833–3835.
(8) Kitamura, M.; Tokunaga, M.; Ohkuma, T.; Noyori, R. *Tetrahedron Lett.* **1991**, *32*, 4163–4166.
(9) Bannwarth, W.; Kung, E. *Tetrahedron Lett.* **1989**, *30*, 4219–4222.
(10) Barta, M.; Urpi, F.; Vilarrasa, J. *Tetrahedron Lett.* **1987**, *47*, 5941–5944.
(11) 3-Oxotetradecanoic acid was synthesized in two steps from *n*-undecyl cyanide and benzyl bromoacetate: (i) as step i in ref 6; (ii) H_2, $Pd(OH)_2$ on C/MeOH.
(12) Krepinsky, J.; Douglas, S.; Whitfield, D. *J. Carbohydr. Chem.* **1993**, *12*, 131–136.
(13) Synthesized in two steps: (i) phenacyl (*R*)-3-oxotetradecanoate (obtained in a similar manner as described ref 6 steps i–iv, starting with *n*-undecyl cyanide), *cis*-Δ^7-tetradecenoic acid[14] DCC/DMAP/CH_2Cl_2/rt; (ii) Zn dust/AcOH/rt.
(14) Normant, A.; Cahiez, G. *Tetrahedron Lett.* **1980**, *21*, 1433–1436.
(15) Synthesized as described in ref 12 starting with *trans*-Δ^7-tetradecenoic acid.[16]
(16) Obtained by isomerization of *cis* acid: Sonnet, P. *J. Org. Chem.* **1980**, *45*, 154–157.
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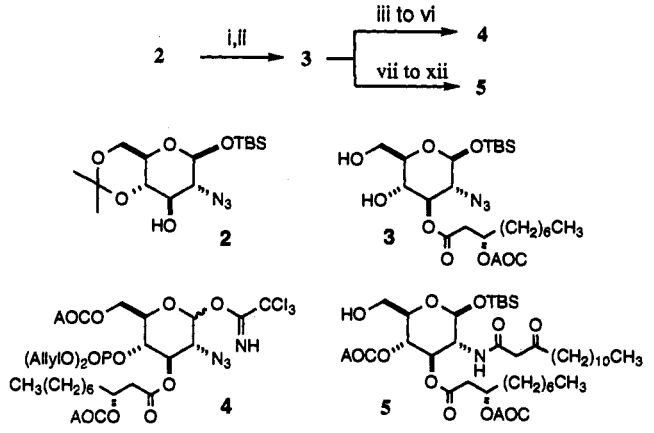
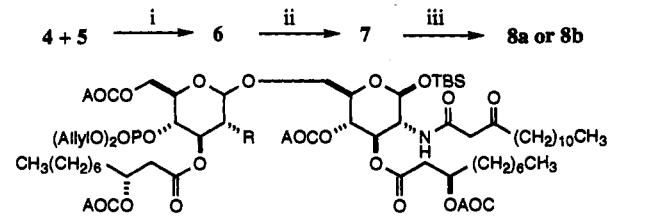
Scheme 1[a]



[a] Reagents and reaction conditions: (i) DCC/DMAP/CH_2Cl_2/(*R*)-3-(((allyloxy)carbonyl)oxy)decanoic acid/0 °C; (ii) AcOH 80%/rt; (iii) allyl chloroformate/toluene/pyridine/0 °C; (iv) bis(allyloxy)(diisopropylamino)phosphine/THF/1*H*-tetrazole/rt then −78 °C/mCPBA; (v) 6 M HF/CH_3CN/rt; (vi) CCl_3CN/K_2CO_3/rt; (vii) TBSCl/imidazole/DMF/0 °C; (viii) phosgene/pyridine/0 °C then allyl alcohol; (ix) tin(II) tris(benzenethiolate)/CH_2Cl_2/rt; (x) 3-oxotetradecanoate/DCC/CH_2Cl_2/rt; (xi) 1 M HF/CH_3CN/rt.

Scheme 2[a]

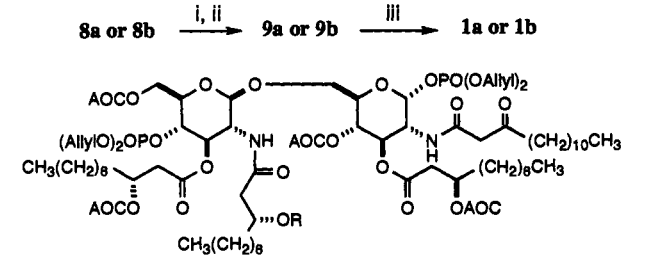


6 R=N_3 **8a** R= Δ^7-*cis*-tetradecenoyl-(R)-3-oxytetradecanoylamino

7 R=NH_2 **8b** R= Δ^7-*trans*-tetradecenoyl-(R)-3-oxytetradecanoylamino

[a] Reagents and reaction conditions: (i) AgOTf/hexanes/rt; (ii) tin(II) tris(benzenethiolate)/CH_2Cl_2/rt; (iii) *cis*- or *trans*-Δ^7-tetradecenoyl-(*R*)-3-oxotetradecanoate/DCC/CH_2Cl_2/rt.

Scheme 3[a]



a series R=Δ^7-*cis*-tetradecenoate

b series R=Δ^7-*trans*-tetradecenoate

[a] Reagents and reaction conditions: (i) 6 M HF/CH_3CN/rt; (ii) bis(allyloxy)(diisopropylamino)phosphine/CH_2Cl_2/1*H*-tetrazole/rt then −78 °C/mCPBA; (iii) THF–AcOH 10:1/$(Ph_3P)_4Pd/Ph_3P$/rt.

to furnish the synthetic *cis*- and *trans*-Rs-DPLA's **1a** and **1b**, respectively, in 90% yield.[19]

Synthetic **1a** and **1b** were compared to several lots of commercially obtained samples of the natural Rs-DPLA[20] (HPTLC, HPLC, and ^{1}H NMR spectroscopy).[21] Chromatographic comparisons of either **1a** or **1b** purified in the same manner[22] as published by Qureshi and co-workers[18] showed no correlation with any of the components of naturally derived Rs-DPLA in either our HPLC system or by HPTLC {[$CHCl_3$:MeOH:AcOH:H_2O, 125:75:10:20 (v/v/v/v)] R_f **1a**, 0.56; **1b**, 0.56; Rs-DPLA, 0.58}. Conversion of **1a**, **1b**, and Rs-DPLA to the tetramethylated[23] derivatives using a procedure described by Qureshi and co-workers[18] clearly showed by HPTLC co-spot **1a** and **1b** to be distinctly different from Rs-DPLA [R_f **1a**, 0.59; **1b**, 0.60; Rs-DPLA appears as two spots at 0.63 and 0.67]. The ^{1}H NMR spectral measurements made at as near identical solvents, concentration, and temperature as possible of synthetic **1a**, **1b**, and Rs-DPLA found a close correspondence. However, small but distinct differences were observed throughout the spectra,[25] leading to the conclusion that the proposed structure for Rs-DPLA could not be completely accurate. Based on our spectral analysis[21] and analog[26] studies, it is felt that the core substitution pattern on the disaccharide backbone is probably correct, but the differences observed may lie in the fatty acid side chains; however, at this point we have not been able to pinpoint these discrepancies. Our efforts were somewhat hampered by the finding that, while both **1a** and **1b** were stable in $CDCl_3/CD_3OD$ for periods of up to 48 h, Rs-DPLA completely decomposed under identical conditions, making some direct spectral measurements impossible. It is worthwhile to note that the olefinic region of the natural Rs-DPLA more closely resembles that of **1a** than that of **1b**, which may suggest the olefinic configuration of natural Rs-DPLA to be *cis*. Finally, an important physical property difference between our synthetic lipid A's and Rs-DPLA was the fact that **1a** and **1b** could be converted to the freely water-soluble stable tetrasodium salts by simple neutralization by NaOH, unlike natural Rs-DPLA, which decomposed upon identical treatment.

Excitingly, both synthetic **1a** and **1b** were found to be as effective as the natural Rs-DPLA for *in vitro* suppression of TNFα generation induced by LPS in human monocytes (average IC_{50} ~ 1 nM). Importantly, unlike the natural Rs-DPLA, the synthetic materials were devoid of agonistic properties in this system even at concentrations as high as 100 μM.[27]

While the absolute structure of Rs-DPLA remains undefined, these unique synthetic antagonists should provide a firm foundation for the examination of the mechanism of action of LPS and the exploration of possible therapeutic intervention in LPS-related disease states. It should be emphasized that the synthetic methods reported have excellent flexibility for the preparation of homogeneous Rs-DPLA-related analogs to study structure–activity relationships.

Acknowledgment. We wish to thank Dr. Kawakami at Eisai Co., Tsukuba, Japan, for mass spectral analysis.

Supplementary Material Available: ^{1}H NMR spectra and selected physical data of Rs-DPLA and compounds **1a** and **1b** (21 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(19) Satisfactory MS and ^{1}H NMR data were obtained for all the compounds reported in this paper.

(20) Purchased from Advanced Medical Research, 8251 Raymond Rd., Madison, WI 53719. HPLC analysis in our system[21] of several different lots of Rs-DPLA purchased displayed varying peak patterns, with the major peak observed at widely different retention times.

(21) Supplied in supplementary material section.

(22) More highly purified samples (>90%) of our synthetic materials can be obtained by reverse-phase HPLC purification. HPLC analysis of our synthetic materials consistently gave reproducible retention times.

(23) The tetramethylated derivatives of **1a** and **1b** were also made and fully characterized using our synthetic methodology, substituting dimethyl *N,N*-diethylphosphoramidate[24] to carry out phosphitylation/phosphorlation in Scheme 1, step iv, and in Scheme 3, step ii.

(24) Kitas, E.; Perich, J.; Tregear, W.; Johns, R. *J. Org. Chem.* **1990**, *55*, 4181–4187.

(25) Major differences noted at 4.2–4.0 and 2.5–2.2 ppm.

(26) Analogs in which the site of the olefin bearing double side-chain attachment was moved to the 2-, 3-, or 3′-positions, the ketoamide-bearing side chain was moved to the 2′-position, or the configurations of the side chains were inverted have also been synthesized, but no matches were found.

(27) Details of biological data to be published elsewhere.