



Efficient Asymmetric Synthesis of (*R*)-3-Hydroxy- and Alkanoyloxytetradecanoic Acids and Method for the Determination of Enantiomeric Purity

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Abstract: An efficient synthesis of the (*R*)-3-hydroxy- and alkanoyloxytetradecanoic acid components of bacterial lipid A has been achieved using a Ru(II)-Binap-catalyzed low-pressure hydrogenation in the key step. The enantiomeric purity of *p*-bromophenacyl ester intermediate 4 could be assessed directly by chiral HPLC—obviating separate derivatization steps and/or chiral NMR shift studies.
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Lipid A constitutes the active principle of lipopolysaccharide (LPS, endotoxin), a complex amphipathic molecule located on the cell surface of Gram-negative bacteria. Both lipid A and LPS elicit not only the typical endotoxic reactions such as fever and lethal shock but also adjuvant, antitumor and other beneficial effects.¹ As part of the synthetic effort aimed at preparing bacterial lipid A (e.g., *Salmonella minnesota* lipid A¹, Figure 1) and analogs possessing a better balance between endotoxic and beneficial immunostimulant activities, several methods have been developed for the synthesis of the (*R*)-3-hydroxy and alkanoyloxy fatty acid components comprising lipid A molecules.²⁻⁶

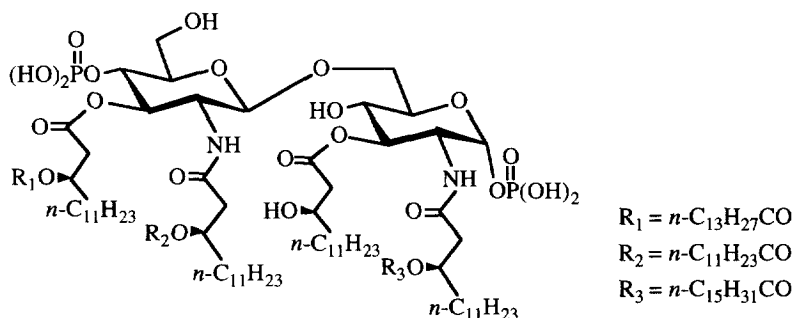
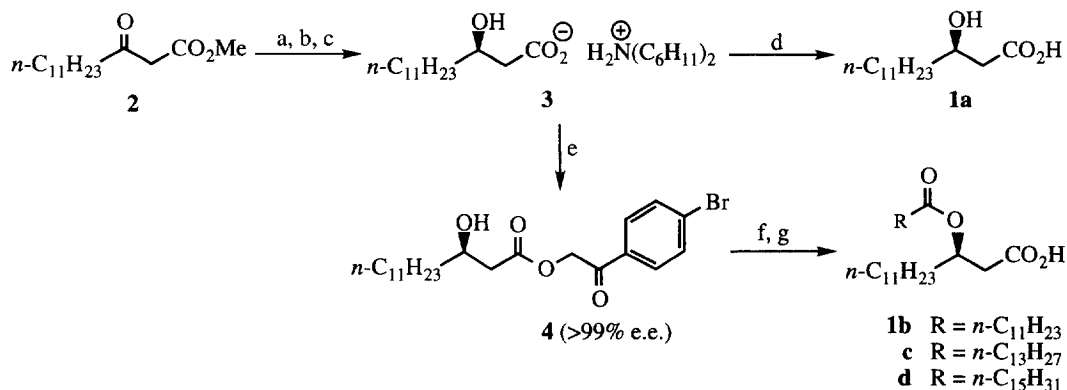


Fig. 1. Lipid A Component of *S. minnesota* Lipopolysaccharide

While the synthesis of (*R*)-3-hydroxytetradecanoic acid derivatives—the most common of the chiral acids present in lipid A—has been achieved by both chemical²⁻⁵ and enzymatic⁶ methods, none of these procedures afford a truly practical method for large-scale preparations. Moreover, the determination of enantiomeric purity by NMR analysis of Mosher MTPA ester derivatives^{2,6b} and/or by chiral NMR shift studies⁵ requires separate derivatization steps and can suffer from kinetic fractionation⁷ during the preparation of diastereomeric derivatives. NMR-based e.e. determinations are also less accurate than chromatographic methods. Here we describe a short, efficient synthesis of the (*R*)-3-hydroxy-, dodecanoyloxy-, tetradecanoyloxy- and hexadecanoyloxytetradecanoic acid components of *S. minnesota* lipid A (**1a-d**, Scheme 1) utilizing a Ru(II)–Binap-catalyzed low-pressure hydrogenation⁸ of readily available methyl 3-oxotetradecanoate **2**^{5a} in the key step and the subsequent determination of enantiomeric purity of scalemic **3** via HPLC analysis of *p*-bromophenacyl ester intermediate **4** using a chiral stationary column.

Asymmetric hydrogenation of keto ester **2** in methanol using commercially available (RuCl₂[(*R*)-Binap])₂•NEt₃ catalyst^{8,9} (~0.05 mol%) on a standard Parr apparatus at 45°C and 60 psig hydrogen in the presence of 0.1 mol% HCl followed by base hydrolysis of the resulting hydroxy ester and dicyclohexylamine (DCHA) treatment furnished DCHA salt **3** in 97% e.e. and 91% chemical yield after crystallization from acetonitrile.^{10,11} The enantiomeric purity of **3**, which could be enhanced to >99% by recrystallization,⁵ was determined by direct conversion of the salt to *p*-bromophenacyl ester **4** and HPLC analysis on a Chiralpak® AS column¹² (detection limit ~99.5% e.e.; R_s~1.5 for racemic **4**; Figure 2). For comparative purposes, the *S* enantiomer of **4** (*ent-4*) was prepared using (*S*)-Binap-derived catalyst in the hydrogenation step. This methodology proved to be highly effective for preparing large quantities (≥100 g) of scalemic salt **3** (or its antipode) and provided a convenient means for accurately assessing enantiomeric purity using intermediate **4** directly. In contrast, the corresponding enantiomeric phenacyl (PAC) esters¹³ could not be resolved by this HPLC method.



Scheme 1: (a) (RuCl₂[(*R*)-Binap])₂•NEt₃/H₂, MeOH, cat. HCl; (b) aq. LiOH/THF, then H⁺; (c) DCHA/CH₃CN, then recryst. from CH₃CN, 78% (3 steps); (d) H⁺, quant.; (e) *p*-BrC₆H₄COCH₂Br, Et₃N, EtOAc, 91%; (f) RCOCl, pyr, CH₂Cl₂; (g) Zn, AcOH, 85–90% (2 steps).

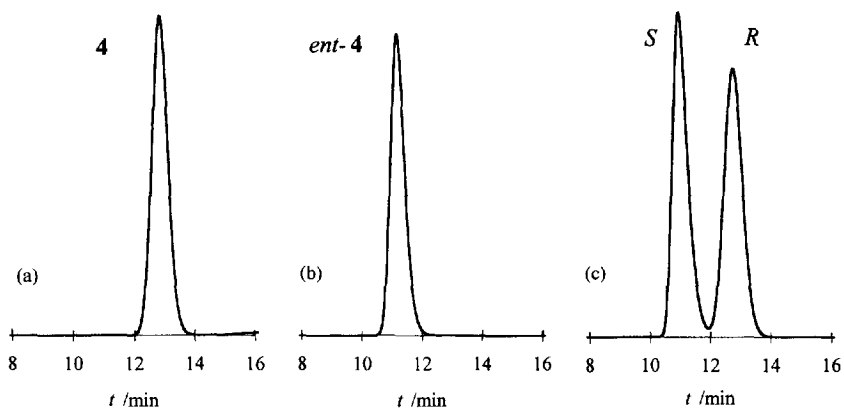


Fig. 2. HPLC Chromatograms of Compound **4** (a), *ent-4* (b) and Racemic Material (c)

The *p*-bromophenacyl ester **4**, which was also found to be more easily purified by crystallization than the PAC ester, was converted to acyloxy acids **1b-d** in 85-90% overall yield by the acylation/deprotection sequence described previously for the PAC ester.¹³ Hydroxy acid **1a** was obtained in quantitative yield from **3** via aqueous acid treatment.

In summary, a practical large-scale synthesis of the (*R*)-3-hydroxy- and alkanoyloxytetradecanoic acid components of bacterial lipid A has been developed along with a convenient HPLC procedure for assessing enantiomeric purity. These methods should find useful application to lipid A analogs and other natural product derivatives containing chiral β -hydroxy- and acyloxytetradecanoic acids.

EXPERIMENTAL

General Methods. Melting points were determined with a Mel-Temp Laboratory Devices apparatus and are uncorrected. Elemental analyses were conducted by Atlantic Microlabs, Norcross, GA. Flash chromatography was performed on Silica Gel 60 (Merck, 230-400 mesh). Optical rotations were determined with a Jasco DIP-1000 digital polarimeter. ¹H and ¹³C NMR spectra were recorded at 300 and 75 MHz, respectively, on a Varian Gemini 300 spectrometer using Me₄Si as an internal standard. The enantiomeric purity of compound **4** was determined with a Waters HPLC system on a Chiral Technologies Chiralpak AS 0.46 x 25 cm column (maintained at 35°C) eluting isocratically with 97:3 (v/v) hexanes-isopropanol at a flow rate of 1.0 ml/min (254 nm detection); *t*_R 12.8 min for compound **4** and 11.1 min for the (*S*)-enantiomer of **4**. All starting materials and reagents were commercially available and used without purification unless otherwise indicated.

Dicyclohexylammonium (*R*)-3-hydroxytetradecanoate (3). A solution of methyl 3-oxotetradecanoate (**2**)^{5a} (100 g, 0.39 mol) in MeOH (500 mL) was degassed with argon (15 min). RuCl₂[(*R*)-Binap]₂•NEt₃ catalyst (0.47 g, 0.28 mmol) and 2 M aq. HCl (2.5 mL) were added and the resulting mixture was hydrogenated on a standard Parr shaker apparatus at 60 psig and 40-50°C for 18 h.¹⁴ The reaction was diluted with hexanes

(300 mL), filtered through a short column of silica gel, and concentrated. A solution of the crude hydroxy ester in THF (200 mL) was treated with 2.4 M aq. LiOH (83 mL) and stirred vigorously at room temperature for 4 h. The resulting slurry was partitioned between ether (300 mL) and 1 M aq. HCl (250 mL) and the layers separated. The aqueous layer was extracted with ether (150 mL) and the combined ethereal extracts were dried (Na_2SO_4) and concentrated. A solution of the crude hydroxy acid in hot acetonitrile (1250 mL) was treated with dicyclohexylamine (85 mL, 0.43 mol) and then heated on a steam bath for 1 h. The DCHA salt (151 g, 91% yield; 97% e.e. by HPLC analysis of derivative **4**) that crystallized upon cooling was collected and recrystallized from acetonitrile (1.3 L) to yield 130 g (78%) of the DCHA salt **3** as a colorless solid: mp 94–95°C (lit.^{5b} mp 94–95°C); $[\alpha]_{\text{D}} -11.3$ (*c* 1.11, CHCl_3); $^1\text{H NMR}$ δ 0.88 (~t, 3H, $J \sim 6.5$ Hz, Me), 1.05–1.58 (m, 24H), 1.65 (m, 2H), 1.80 (m, 4H), 2.01 (br d, 4H), 2.18 (dd, 1H, $J_{\text{AB}} = 15.7$ Hz, $J_{\text{BX}} = 9.4$ Hz, H-2_b), 2.36 (dd, 1H, $J_{\text{AB}} = 15.7$ Hz, $J_{\text{AX}} = 2.6$ Hz, H-2_a), 2.94 (m, 2H, H-1 of DCHA), 3.84 (m, 1H, H-3); $^{13}\text{C NMR}$ δ 14.2, 22.7, 24.9, 25.1, 25.3, 25.7, 29.5, 29.7, 29.8, 32.0, 37.2, 43.5, 52.7, 68.6, 178.8.

ent-**3**, prepared in 85% yield by the above method using $\text{RuCl}_2[(S)\text{-Binap}]_2\cdot\text{NEt}_3$ catalyst⁹, exhibited ^1H and $^{13}\text{C NMR}$ spectra identical to DCHA salt **3**. Data for *ent*-**3**: mp 94–95°C; $[\alpha]_{\text{D}} +11.9$ (*c* 1.5, CHCl_3).

(R)-3-Hydroxytetradecanoic acid (1a). Acidification of the DCHA salt **3** with 1 M aq. HCl and ether extraction gave a quantitative yield of the free acid **1a** as a colorless solid: mp 72–73°C (lit.^{5a} mp 72.0°C, lit.^{6b} mp 72.0–72.5°C, lit.^{6c} mp 71.0–71.5°C); $^1\text{H NMR}$ δ 0.88 (~t, 3H, $J \sim 6.5$ Hz, Me), 1.15–1.65 (m, 20H), 2.47 (dd, 1H, $J_{\text{AB}} = 16.5$ Hz, $J_{\text{BX}} = 8.8$ Hz, H-2_b), 2.58 (dd, 1H, $J_{\text{AB}} = 16.5$ Hz, $J_{\text{AX}} = 3.3$ Hz, H-2_a), 4.03 (m, 1H, H-3); $^{13}\text{C NMR}$ δ 14.1, 22.7, 25.5, 29.4, 29.5, 29.6, 31.9, 36.5, 41.1, 68.1, 177.8.

***p*-Bromophenacyl (R)-3-hydroxytetradecanoate (4)**. A solution of **3** (50 g, 0.117 mol) and 2,4'-dibromoacetophenone (39 g, 0.14 mol) in EtOAc (2.3 L) was treated with triethylamine (19.6 mL, 0.14 mol), and the resulting solution was stirred for 18 h at room temperature. The voluminous precipitate that formed was collected and triturated with warm EtOAc (3 x 400 mL). The combined triturates and filtrate were washed with 1 M aq. HCl, saturated aq. NaCl, and dried (Na_2SO_4). Volatiles were removed under reduced pressure and the crude product obtained was crystallized from EtOAc-hexanes to give 47.2 g (91% yield, >99% e.e.) of **4** as a colorless solid: mp 109–109.5°C; $[\alpha]_{\text{D}} = -2.4$ (*c* 2.39, CHCl_3); $^1\text{H NMR}$ δ 0.88 (~t, 3H, $J \sim 6.5$ Hz, Me), 1.15–1.70 (m, 20H), 2.56 (dd, 1H, $J_{\text{AB}} = 15.1$ Hz, $J_{\text{BX}} = 9.1$ Hz, H-2_b), 2.69 (dd, 1H, $J_{\text{AB}} = 15.1$ Hz, $J_{\text{AX}} = 2.9$ Hz, H-2_a), 3.27 (br s, 1H, OH), 4.12 (m, 1H, H-3), 5.31 (d, 1H, $J_{\text{AB}} = 16.5$ Hz, $-\text{CO}_2\text{CH}_b-$), 5.42 (d, 1H, $J_{\text{AB}} = 16.5$ Hz, $-\text{CO}_2\text{CH}_a-$), 7.65 (d, 2H, $J_{\text{AB}} = 8.5$ Hz, ArH), 7.78 (d, 2H, $J_{\text{AB}} = 8.5$ Hz, ArH); $^{13}\text{C NMR}$ δ 14.1, 22.7, 25.6, 29.4, 29.6, 31.9, 36.6, 42.0, 65.8, 68.4, 129.2, 129.5, 132.5, 171.8, 191.5. Anal. calcd for $\text{C}_{22}\text{H}_{33}\text{O}_4\text{Br}$: C, 59.86; H, 7.54; Br, 18.10. Found: C, 59.96; H, 7.54; Br, 17.98.

ent-**4**, prepared in 85% yield (>99% e.e.) by the above method, exhibited ^1H and $^{13}\text{C NMR}$ spectra identical to compound **4**. Data for *ent*-**4**: mp 108–109°C; $[\alpha]_{\text{D}} +2.5$ (*c* 2.18, CHCl_3); Anal. calcd for $\text{C}_{22}\text{H}_{33}\text{O}_4\text{Br}$: C, 59.86; H, 7.54; Br, 18.10. Found: C, 59.88; H, 7.53; Br, 18.04.

(R)-3-Tetradecanoyloxytetradecanoic acid (1c). A solution of **4** (10.0 g, 22.6 mmol) in CH₂Cl₂ (150 mL) containing pyridine (13.4 mL, 165 mmol) was treated dropwise at room temperature with myristoyl chloride (7.5 mL, 27.7 mmol). After stirring overnight at room temperature, MeOH (5 mL) was added and the reaction mixture was stirred for an additional 0.5 h and then concentrated. The residue was partitioned between EtOAc and 1 M aq. HCl and the layers separated. The organic layer was washed with saturated aq. NaCl and dried (Na₂SO₄). Volatiles were removed under reduced pressure and the crude product obtained was used without further purification. (Purification of a separate sample by flash chromatography on silica gel with 1:19 (v/v) EtOAc-hexanes and crystallization from EtOAc-hexanes afforded pure *p*-bromophenacyl (R)-3-tetradecanoyloxytetradecanoate (95%) as a colorless solid: mp 44-45°C; ¹H NMR δ 0.88 (~t, 6H, Me), 1.15-1.40 (m, 38H), 1.50-1.75 (m, 4H), 2.30 (t, 2H, *J* = 7.5 Hz, H-2'), 2.70 (dd, 1H, *J*_{AB} = 15.3 Hz, *J*_{BX} = 5.5 Hz, H-2_b), 2.77 (dd, *J*_{AB} = 15.3 Hz, *J*_{AX} = 7.2 Hz, H-2_a), 5.23-5.34 (m, 3H, H-3 and —CO₂CH₂—), 7.63 (d, 2H, *J*_{AB} = 8.4 Hz, ArH), 7.76 (d, 2H, *J*_{AB} = 8.4 Hz, ArH); ¹³C NMR δ 14.2, 22.7, 25.0, 25.2, 29.2, 29.4, 29.6, 29.7, 30.1, 31.9, 34.0, 34.5, 39.0, 65.9, 70.1, 129.2, 132.2, 132.8, 169.8, 173.2, 190.8.) A solution of the crude acyloxy ester in AcOH (85 mL) at 60°C was treated with three equal portions of zinc dust (35 g total, 0.54 mol) over 0.5 h and then stirred for an additional 0.5 h at 60°C. The cooled reaction mixture was sonicated briefly, filtered through Celite® and concentrated. The residue was purified by flash chromatography on silica gel (gradient elution, 0→3% MeOH-CHCl₃) to give 9.1 g (88% from **4**) of compound **1c** as a colorless solid: mp 28-29°C (lit.² mp 28-29°C; lit.¹³ mp 38.5-40°C); ¹H NMR δ 0.88 (~t, 6H, Me), 1.15-1.40 (m, 38H), 1.50-1.70 (m, 4H), 2.28 (t, 2H, *J* = 7.4 Hz, H-2'), 2.56 (dd, 1H, *J*_{AB} = 15.9 Hz, *J*_{BX} = 5.8 Hz, H-2_b), 2.63 (dd, 1H, *J*_{AB} = 15.9 Hz, *J*_{AX} = 7.1 Hz, H-2_a), 5.21 (m, 1H, H-3); ¹³C NMR δ 14.2, 22.7, 25.1, 25.2, 29.2, 29.3, 29.4, 29.5, 29.7, 32.0, 34.0, 34.5, 40.0, 70.1, 173.3, 175.7.

Compounds **1b** and **1d**, obtained as described for **1c** above using lauroyl and palmitoyl chloride, respectively, in lieu of myristoyl chloride, exhibited ¹H and ¹³C NMR spectra nearly identical to those of **1c** and in agreement with reported spectral data.^{2,3a}

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REFERENCES AND NOTES

1. Takada, H.; Kotani, S. *Crit. Rev. Microbiol.* **1989**, *16*, 477-523.
2. Oikawa, M.; Kusumoto, S. *Tetrahedron: Asymmetry* **1995**, *6*, 961-966 and references cited therein.
3. (a) Kamireddy, B.; Darsley, M.J.; Simpson, D.M.; Massey, R.J. PCT Int. Pat. Appl., **1993**, WO 93/19761. (b) For the preparation of **3** via catalytic asymmetric hydrogenation⁴ of ethyl 3-oxotetradecanoate at 1480 psig, see: Christ, W.J.; Kawata, T.; Hawkins, L.D.; Kobayashi, S.; Asano, O.; Rossignol, D.P. Eur. Pat. Appl., **1993**, EP 0536,969 A2; Christ, W.J.; McGuinness, P.D.; Asano, O.; Wang, Y.; Mullarkey, M.A.; Perez, M.; Hawkins, L.D.; Blythe, T.A.; Dubuc, G.R.; Robidoux, A.L. *J. Am. Chem. Soc.* **1994**, *116*, 3637-3638.

4. Kitamura, M.; Tokunaga, M.; Ohkuma, T.; Noyori, R. *Tetrahedron Lett.* **1991**, *32*, 4163-4166 and references cited therein.
5. (a) Nakahata, M.; Imaida, M.; Ozaki, H.; Harada, T.; Tai, A. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2186-2189. (b) Tai, A.; Nakahata, M.; Harada, T.; Izumi, Y.; Kusumoto, S.; Inage, M.; Shiba, T. *Chem. Lett.* **1980**, 1125-1126.
6. (a) Fukase, K.; Liu, W.-C.; Suda, Y.; Oikawa, M.; Wada, A.; Mori, S.; Ulmer, A.J.; Rietschel, E.T.; Kusumoto, S. *Tetrahedron Lett.* **1995**, *36*, 7455-7458. (b) Sugai, T.; Ritzén, H.; Wong, C.-H. *Tetrahedron: Asymmetry* **1993**, *4*, 1051-1058. (c) Utaka, M.; Watabu, H.; Higashi, H.; Sakai, T.; Tsuboi, S.; Torii, S. *J. Org. Chem.* **1990**, *55*, 3917-3921.
7. Dutcher, J.S.; Macmillan, J.G.; Heathcock, C.H. *J. Org. Chem.* **1976**, *41*, 2663-2669.
8. (a) King, S.A.; Thompson, A.S.; King, A.O.; Verhoeven, T.R. *J. Org. Chem.* **1992**, *57*, 6689-6691. (b) Taber, D.F.; Silverberg, L.J. *Tetrahedron Lett.* **1991**, *32*, 4227-4230.
9. Strem Chemicals, Inc., Newburyport, MA.
10. For a recent synthesis of methyl (*S*)-3-hydroxyoctadecanoate from the corresponding β -keto ester using *in situ* prepared $(\text{RuBr}_2[(S)\text{-Binap}])_2 \cdot \text{Me}_2\text{CO}$ catalyst (2 mol%), see: Genêt, J.P.; Ratovelomanana-Vidal, V.; Caño de Andrade, M.C.; Pfister, X.; Guerreiro, P.; Lenoir, J.Y. *Tetrahedron Lett.* **1995**, *36*, 4801-4804.
11. For a related low-pressure hydrogenation of methyl 3-oxotetradecanoate (**2**) using a novel bis(phospholane)-Ru catalyst, see: Burke, M.J.; Harper, T.G.P.; Kalberg, C.S. *J. Am. Chem. Soc.* **1995**, *117*, 4423-4424.
12. Chiral Technologies, Inc., Exton, PA.
13. Kiso, M.; Tanaka, S.; Fujita, M.; Fujishima, Y.; Ogawa, Y.; Hasegawa, A. *Carbohydr. Res.* **1987**, *162*, 247-256 and references cited therein.
14. Ethyl 3-oxotetradecanoate, prepared via Wierenga's method¹⁵ or the Blaise reaction^{3b}, can be substituted for keto ester **2** without a decrease in enantioselectivity.
15. Wierenga, W.; Skulnick, H.I. *J. Org. Chem.* **1979**, *44*, 310-311.

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