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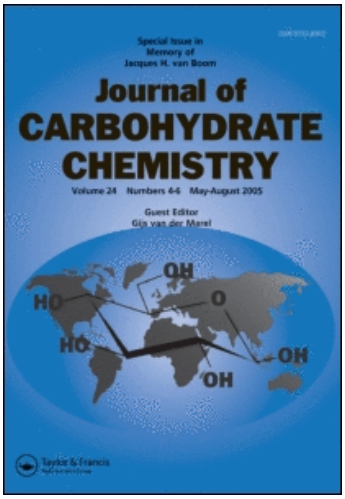

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
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To cite this Article Johnson, David A. , Sowell, C. Gregory , Keegan, David S. and Livesay, Mark T.(1998) 'Chemical Synthesis of the Major Constituents of *Salmonella Minnesota* Monophosphoryl Lipid A', Journal of Carbohydrate Chemistry, 17: 9, 1421 — 1426
To link to this Article: DOI: 10.1080/07328309808002363
URL: http://dx.doi.org/10.1080/07328309808002363

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J. CARBOHYDRATE CHEMISTRY, 17(9), 1421-1426 (1998)

COMMUNICATION

CHEMICAL SYNTHESIS OF THE MAJOR CONSTITUENTS OF *SALMONELLA MINNESOTA* MONOPHOSPHORYL LIPID A

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Received April 29, 1998 - Final Form July 1, 1998

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Structural investigations have shown that lipid A constitutes the active principle of lipopolysaccharide (LPS, endotoxin), a complex amphipathic molecule located on the cell surface of Gram-negative bacteria.[1] Lipid A elicits not only the typical endotoxic reactions such as fever and lethal shock but also adjuvant, antitumor and other beneficial effects.[1] As a result, there has been a great deal of interest in the synthesis of lipid A derivatives possessing low toxicity.[1,2]

Recently, it was demonstrated that the endotoxic effects of the lipid A from *Salmonella minnesota* R595 (**1**) could be ameliorated by selective hydrolysis of the 1-phosphate[3] and (*R*)-3-hydroxytetradecanoate[4] groups. *S. minnesota* lipid A modified in this way is an effective adjuvant in both prophylactic and therapeutic human vaccines.[4b] However, the steps involved in the isolation and modification of **1** (or the cognate LPS) can lead to undesired side reactions, resulting in heterogeneous monophosphoryl lipid A preparations.[5] Structural analysis of the monophosphoryl lipid A derived from Re mutants of *S. minnesota* R595 (MLA) indicates that two of the major components present in MLA are disaccharides **2** and **3**.[6] To confirm the chemical structure of these putative components and the postulate that the biological activity of MLA is due primarily to these materials and not to the presence of other bioactive substances, we

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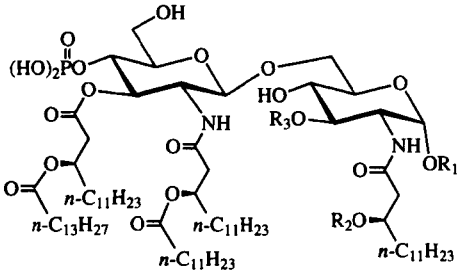
undertook the independent synthesis of compounds **2** and **3**. Here we report the first total synthesis of these *S. minnesota* R595 monophosphoryl lipid A constituents.



	R_1	R_2	R_3
1	PO_3H_2	*n*-$C_{15}H_{31}CO$	*n*-$C_{11}H_{23}$
2	H	H	H
3	H	*n*-$C_{15}H_{31}CO$	H

Our strategy (Schemes 1 and 2) for the synthesis of **2** and **3** utilizes the *N*-2,2,2-trichloroethoxycarbonyl (Troc) method[7] and silver ion catalysis[7b] to construct the β-(1→6) disaccharide linkage in a stereospecific manner from glycosyl chloride **13** and diols **7** and **8**. The 2-(trimethylsilyl)ethyl (TMSEt, SE) group was chosen for anomeric protection in the synthesis of glycosyl chloride **13** because of the facility with which the TMSEt group can be converted directly into 1-chloro derivatives.[8]

Glycosyl acceptors **7** and **8** were synthesized as shown in Scheme 1. *N*-Acylation of **4**[9] with (*R*)-3-hydroxy- or hexadecanoyloxytetradecanoic acid[10] in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (EDC·MeI) furnished amides **5** (95%, mp 104.5-106.5 °C; lit.[9] mp 99-102 °C) and **6** (88%, mp 69-71 °C, FAB-MS[11] $[M+H]^+$ *m/z* 774). The ^1H NMR spectrum[12] of **6** revealed salient ring-proton signals at δ 4.69 (d, *J*=8.3 Hz, H-1), 3.61 (~t, *J*~9 Hz, H-3), 3.51 (m, H-2), and 3.30 (~td, *J*~5, 10 Hz, H-5). Hydroxyl protection with 2,2,2-trichloroethyl chloroformate (Troc-Cl) and acetonide hydrolysis then gave **7** (96%, mp 113.5-115 °C, $[M+H]^+$ *m/z* 844) and **8** (93%, amorphous solid, $[M+H]^+$ *m/z* 908). The ^1H NMR spectra of **7** and **8** exhibited nearly identical AB patterns for the methylene protons α to the amide carbonyl; data for **8**: δ 2.43, 2.29 (2 dd, 1 and 1H, *J*=5, 14.7 Hz).

The synthesis of glycosyl donor **13** and glycosylation of **7** and **8** are presented in Scheme 2. *N*-Protection of **9**[13] under Schotten-Baumann conditions with Troc-Cl afforded **10** (96%, mp 72 °C, $[M+H]^+$ *m/z* 516). Significant signals in the ^1H NMR spectrum of **10** appeared at δ 4.74 (m, AB type, CH_2CCl_3), 4.65 (d, *J*=8.3 Hz, H-1), and 3.79 (~t, *J*~10 Hz, H-6_{ax}). 3-*O*-Acylation with (*R*)-3-tetradecanoyloxytetradecanoic acid[10] in the presence of EDC·MeI and 4-pyrrolidinopyridine followed by acetonide cleavage gave diol **11** (83%, amorphous solid, $[M+Na]^+$ *m/z* 912). Notable absorptions in the ^1H NMR spectrum of **11** included a doublet at δ 4.57 (*J*=8.3 Hz, H-1) and a two-

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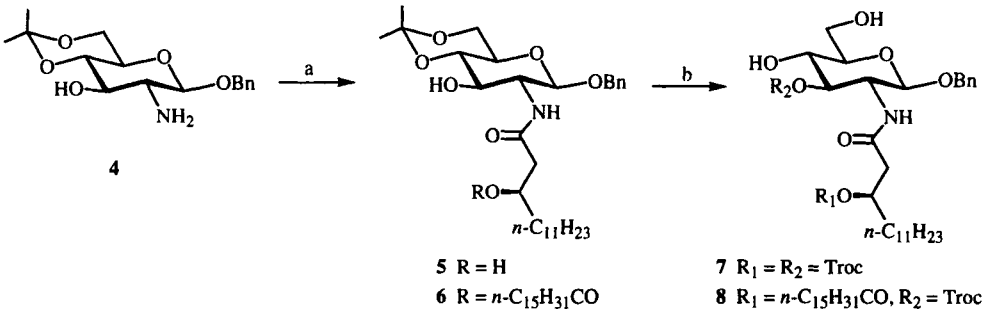

Scheme 1: (a) (*R*)-3-hydroxy- or hexadecanoyloxytetradecanoic acid, EDC•MeI, CH_2Cl_2; (b)Troc-Cl, DMAP, pyridine, CH_2Cl_2; then 80% aq. AcOH, 60 °C.

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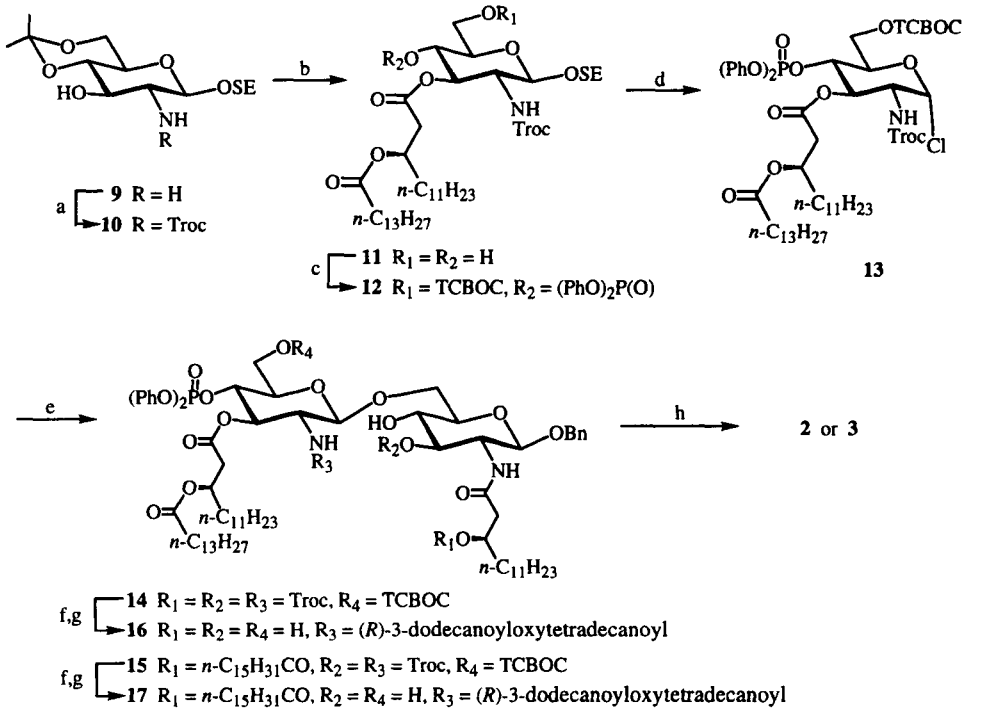

Scheme 2: (a) Troc-Cl, 1N aq. $NaHCO_3$, CH_2Cl_2; (b) (*R*)-3-tetradecanoyloxytetradecanoic acid, 4-pyrrolidinopyridine, EDC•MeI, CH_2Cl_2; then 90% aq. AcOH, 60 °C; (c) TCBOC-Cl, pyridine, CH_2Cl_2; then $(PhO)_2P(O)Cl$, 4-pyrrolidinopyridine, $(i\text{-}Pr)_2NEt$; (d) Cl_2CHOMe, $ZnCl_2$ (cat.), $CHCl_3$; (e) **7** or **8**, AgOTf, 4Å sieves, $ClCH_2CH_2Cl$; (f) Zn, AcOH, 60 °C; (g) (*R*)- 3-dodecanoyloxytetradecanoic acid, EEDQ, CH_2Cl_2; (h) Pd black, AcOH, THF, 70 psig H_2; then PtO_2, 70 psig H_2.

proton multiplet at δ 5.0-5.15 (H-3 and —$COCH_2CH$(OAcyl)—). The greater primary selectivity of 1,1-dimethyl-2,2,2-trichloroethyl chloroformate (TCBOC-Cl) *vis-à-vis* Troc-Cl[7a] was then exploited for the one-pot synthesis of **12** by successive treatment of **11** with TCBOC-Cl and diphenyl chlorophosphate to give donor progenitor **12** (amorphous solid, $[M+Na]^+$ *m/z* 1346) in 95% yield.[14] The ^1H NMR spectrum of **12** showed a characteristic quartet at δ 4.66 (*J*~9 Hz) for H-4 due to nearly equal vicinal proton and three-bond $^1H/^{31}P$ coupling constants. Other prominent signals appeared at δ 5.21 (m, —$COCH_2CH$(OAcyl)—), 5.03 (d, *J*=7.9 Hz, H-1), 4.34 (d, *J*=12 Hz, H-6a), and 4.27 (dd, *J*=5, 12 Hz, H-6b). Transformation of the TMSEt glycoside **12** into the chromatographically stable glycosyl chloride **13** (88%, amorphous solid, $[M+Na]^+$ *m/z* 1264) was accomplished directly with α,α-dichloromethyl methyl ether/zinc chloride[8] in chloroform. The ^1H NMR spectrum of **13** showed the disappearance of TMSEt signals and revealed a doublet at δ 6.26 (*J*=3.6 Hz, H-1) indicative of the α-chloride, and other ring-proton signals at δ 5.51 (~t, *J*~9 Hz, H-3) and 4.83 (~q, *J*~9 Hz, H-4).

Koenigs-Knorr coupling of chloride **13** (1.2 equiv) with **7** or **8** was effected in the presence of silver triflate at room temperature to give exclusively the β-disaccharides **14** (79%, mp 70.5-73.5 °C, $[M+Na]^+$ *m/z* 2071) and **15** (73%, mp 95-97 °C, $[M+Na]^+$ *m/z* 2136).[15] The β-(1→6) linkage was assured by 300 MHz ^1H-^1H COSY experiments and by analogy with earlier work.[7a] The ^1H NMR spectra of **14** and **15** were virtually identical with respect to the AB patterns for the H-6′ and H-6 protons; data for **15**: δ 4.38 (d, *J*=11 Hz, H-6′a), 4.24 (dd, *J*=5, 11 Hz, H-6′b), 4.17 (dd, *J*=2.5, 12 Hz, H-6a), and 3.88 (dd, *J*=3.5, 12 Hz, H-6b). Reductive cleavage of the trichloroethyl-based protecting groups with zinc dust in acetic acid and *N*-acylation with (*R*)-3-dodecanoyloxytetradecanoic acid[10] in the presence of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) furnished disaccharides **16** (61%, $[M+Na]^+$ *m/z* 1756) and **17** (51%, $[M+Na]^+$ *m/z* 1994) as resinous solids. The ^1H NMR spectra for **16** and **17** displayed doublets at δ 4.55 (*J*=7.6 Hz) and 4.54 (*J*=7.8 Hz), respectively, for the H-1 protons. Final elaboration of **2** (54%, mp 171-173 °C (dec)) and **3** (65%, 195-196 °C (dec)), isolated as triethylammonium salts, was accomplished by hydrogenolytic deprotection[7a] and lyophilization of the free acids (formed by Bligh-Dyer extraction)[16] from 1% aqueous triethylamine. The ^1H NMR spectra (1:1 $CDCl_3$–CD_3OD) for **2** and **3** showed the anomeric protons of the β-glycosides at δ 4.63 (d, *J*=8.5 Hz) and 4.66 (d, *J*=8.0 Hz), respectively, and those of the reducing sugars at δ 5.11 (d, *J*=3.8 Hz) and 5.05 (d, *J*=3.3 Hz), indicating that the α-D-pyranose form of **2** and **3** predominates in solution. Negative-ion FAB-MS of synthetic **2** and **3** gave characteristic[17] $[M-H]^-$ quasi-molecular ion peaks at *m/z* 1490 and 1728, respectively.

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Reverse-phase HPLC comparison[18] of the synthetic and natural materials derivatized with 3,5-dinitrobenzyloxyamine using spiking experiments showed a direct correlation of synthetic **2** and **3** with the two MLA components whose structures had been established previously by fractionation and FAB-MS.[6] Preliminary comparison of biological activities (pyrogenicity, lethal toxicity and macrophage activation)[1,4,19] indicate that synthetic **2** and **3** exhibit immunostimulant activities comparable to those of the natural MLA preparation.[20]

In summary, the chemical synthesis of monophosphoryl lipid A compounds **2** and **3** from glycosyl chloride **13** and diols **7** and **8** has permitted structural confirmation of two major components present in *S. minnesota* MLA. Biological evaluation of synthetic **2** and **3** verifies that compounds of this type are responsible for the biological activity of MLA.

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ACKNOWLEDGEMENTS

The authors are grateful to Ms. Kara D. Looysen and Mr. Craig L. Johnson for their excellent technical assistance, and to Dr. William J. Christ (Eisai Research Institute) for suggesting the deuterium pre-exchange technique in the ^{1}H NMR analysis of **2** and **3**.

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11. With the exception of compounds **2** and **3** which were analyzed in the negative-ion mode, all new compounds were analyzed by positive-ion FAB-MS; the observed isotopic distributions for polychloro compounds **7**, **8**, and **10-15** were in agreement with theoretical contributions in terms of intensity of the (quasi-M+2, -M+4, etc.) isotope peaks relative to the quasi-molecular ions.
12. All ^{1}H NMR spectra were obtained at 300 MHz in $CDCl_3$ unless otherwise indicated; chemical shifts (δ) are referenced to tetramethylsilane. The amide and hydroxyl protons of compounds **2** and **3**, and adventitious water were pre-exchanged with deuterium (methanol-d_4) prior to obtaining the ^{1}H NMR spectra.
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15. A typical procedure is as follows. Preparation of **15**: A solution of **13** (2.33 g, 1.85 mmol) and **8** (1.40 g, 1.54 mmol) in 1,2-dichloroethane (18.5 mL) was stirred with powdered 4Å molecular sieves (1 g) for 1 h and then treated with AgOTf (1.43 g, 5.55 mmol) in one portion. After stirring for 4 h at rt in the dark, the reaction mixture was treated with additional AgOTf (0.475 g, 1.85 mmol) and stirred overnight. The resulting slurry was filtered and concentrated. Flash chromatography on silica gel (gradient elution, 20→25% EtOAc-hexanes) afforded 2.40 g (73%) of **15** as a colorless solid.
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