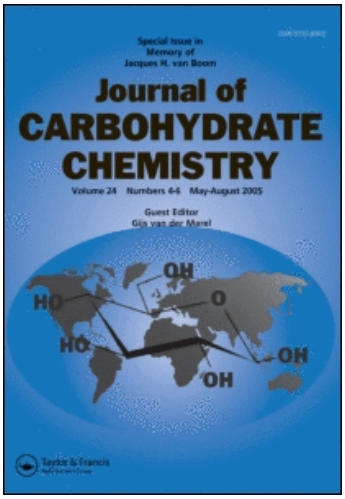

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AN APPROACH TO THE SYNTHESIS OF FOUR *RHODOMICROBIUM VANNIELII* LIPID A ANALOGUES

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ABSTRACT

The preparation of four *Rh. Vannielii* Lipid A analogues (*i.e.* compounds **22**, **23**, **30** and **33**) is described. Non-neighbouring group supported introduction of the β-glycosidic linkages was performed by coupling the mannopyranosyl bromide **2** and the 2-azido-2-deoxy-glucopyranosyl bromides **10** and **13** with the suitably protected glycosyl acceptors **3**, **4** and **5** in the presence of a heterogeneous silver catalyst, to give compounds **6**, **7**, **14** and **24**, respectively. Selective removal of the allyl group and reduction of the azido functions followed by several O,N-acylation steps afforded, after complete deblocking, the tri- and disaccharide *Rh. Vannielii* Lipid A analogues **22**, **23**, **30** and **33**.

INTRODUCTION

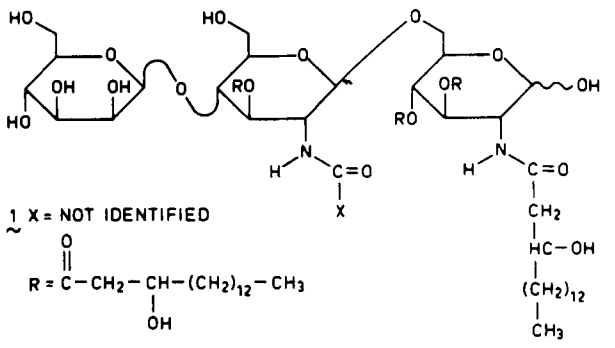
Lipopolysaccharide (LPS), an amphiphatic macromolecule in the outer membrane of Gram-negative bacteria, manifests O-antigenic and endotoxic activities and plays an important role in the pathogenesis of Gram-negative bacterial infections.[1-3] A number of studies demonstrated that Lipid A, the lipophilic moiety of LPS, is responsible for most of the biological properties including pyrogenicity, mitogenicity,

macrophage activation and lethal toxicity.[4-7] Moreover, detoxified fractions of Lipid A may cause tumor regression[7,8]. The Lipid A's of Gram-negative bacteria, e.g. *Salmonella* species and *Escherichia coli*, consist of a β(1-6)-linked D-glucosamine disaccharide substituted by phosphate groups and ester- and amide-bound fatty acids.[7,9] Several analogues of Lipid A have recently been synthesized by several groups.[10-18]

In 1983, Holst et al.[19] reported the structure of a low toxic Lipid A fragment of *Rhodomicrobium Vannielii*, which lacked covalently linked phosphate groups, but contained instead a β-D-mannopyranosyl residue (see compound 1). In this study we report[20] the synthesis of *Rh. Vannielii* Lipid A analogues **22**, **23**, **30** and **33**, by using the properly protected synthons 1,6-anhydro-2-azido-2-deoxy-D-glucopyranoside and 2,3,4-tri-*O*-benzyl-D-mannopyranose. The β-glycosidic bonds in the compounds under investigation were introduced in coupling reactions promoted by insoluble silver salts.

RESULTS AND DISCUSSION

In view of the structural features of the target molecules, we reasoned that application of a heterogeneous silver salt as a promoter for β-glycosylation reactions would be advantageous. The synthesis of the appropriate protected monosaccharides **2-5** was accomplished taking into account substituent effects which determine the stereochemical outcome of a coupling reaction promoted by a heterogeneous silver salt.[21-24] Particularly, the application of a 2-azido-2-deoxy-D-glucose derivative is favourable in our strategy: the azido function at C-2 enhances the β/α ratio in



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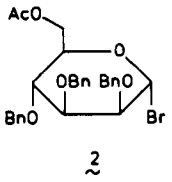

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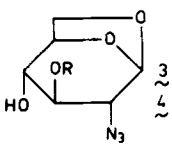
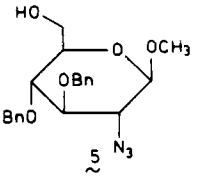


3 : R = Bn
4 : R = Allyl



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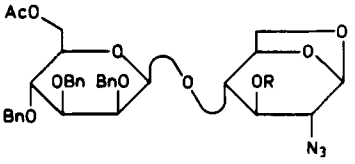
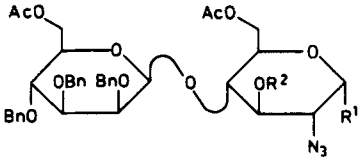
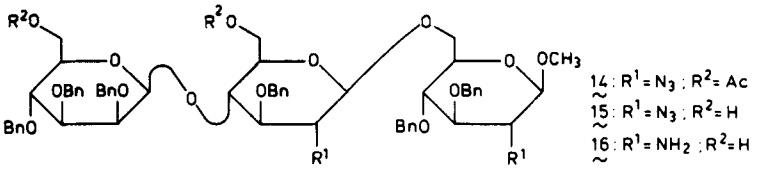


6 : R = Bn
7 : R = Allyl



8 : R^1 = OAc , R^2 = Bn
9 : R^1 = OH , R^2 = Bn
10 : R^1 = Br , R^2 = Bn
11 : R^1 = OAc , R^2 = Allyl
12 : R^1 = OH , R^2 = Allyl
13 : R^1 = Br , R^2 = Allyl



14 : R^1 = N_3 ; R^2 = Ac
15 : R^1 = N_3 ; R^2 = H
16 : R^1 = NH_2 ; R^2 = H

coupling reactions promoted by a heterogeneous silver salt[23] and can be selectively reduced to an amino function under mild conditions. In addition, introduction of the 3-hydroxy-tetradecanoyl amido function can than be performed in a later stage of the synthesis, thus avoiding side reactions during the activation of the anomeric centre in the coupling process.[25,26]

Synthesis of the monosaccharide derivatives 2-5. 6-O-Acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl bromide **2**[27] was prepared in three steps from 1,6-anhydro-β-D-mannopyranose.[28] Synthesis of the 2-azido-2-deoxy-D-glucopyranosides **3-5** was accomplished according to Paulsen et al.[29,30] starting from 1,6:2,3-di-anhydro-β-D-mannopyranose.[31]

Introduction of the β-glycosidic bonds, synthesis of compounds 14 and 24. Glycosylation of **3** with bromide **2** in the presence of silver silicate[32] at -50°C in a mixture of toluene and dichloromethane afforded disaccharide **6**[33] in 80% yield in a β/α ratio of 9/1. Similar results were obtained using **4** as aglycon, giving disaccharide **7**. The ^{13}C and ^{1}H NMR spectra of **7** could be completely assigned by two-dimensional NMR techniques (see FIG. 1 and 2A and B). The ^{13}C resonance at 98.9 ppm with J_{CH} 154 Hz confirms the presence of a β-mannosidic bond.[34]

Acetolysis of **6** and **7** with acetic anhydride/trifluoroacetic acid[35] afforded the corresponding 1,6-diacetates **8** and **11**, respectively. Hydrolysis of the anomeric acetate ester with piperidine in tetrahydrofuran[36] yielded the hydroxy compounds **9** and **12**, which were converted into their α-glycosyl bromides **10** and **13** with oxalyl bromide/*N*,*N*-dimethylformamide[37]. Condensation of **10** with the glycosyl acceptor **5**, as described above, resulted in the exclusive formation of tri-

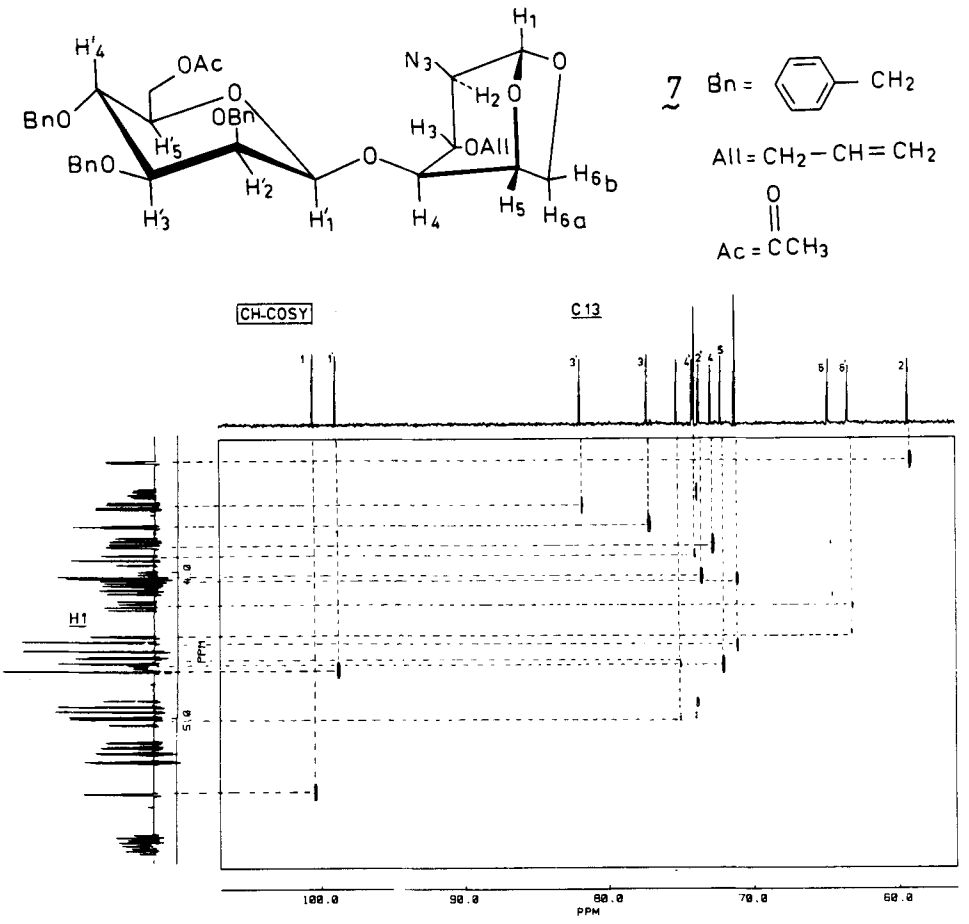


FIG. 1. ^{13}C-^{1}H Heteronuclear correlated NMR spectrum of compound 7.

saccharide 14 in 70% yield. Glycosylation of benzyl alcohol with bromide 13, under identical conditions, afforded the key β-glycoside 24 in 75% yield.

Preparation of Lipid A derivatives 22 and 23. We now, having key trisaccharide 14 at our disposal, turned our attention to the introduction of the *O,N*-acyl functions. Zemplén deacetylation of 14 furnished 15 in a quantitative yield. Selective reduction of the azido functions of 15 was effected by

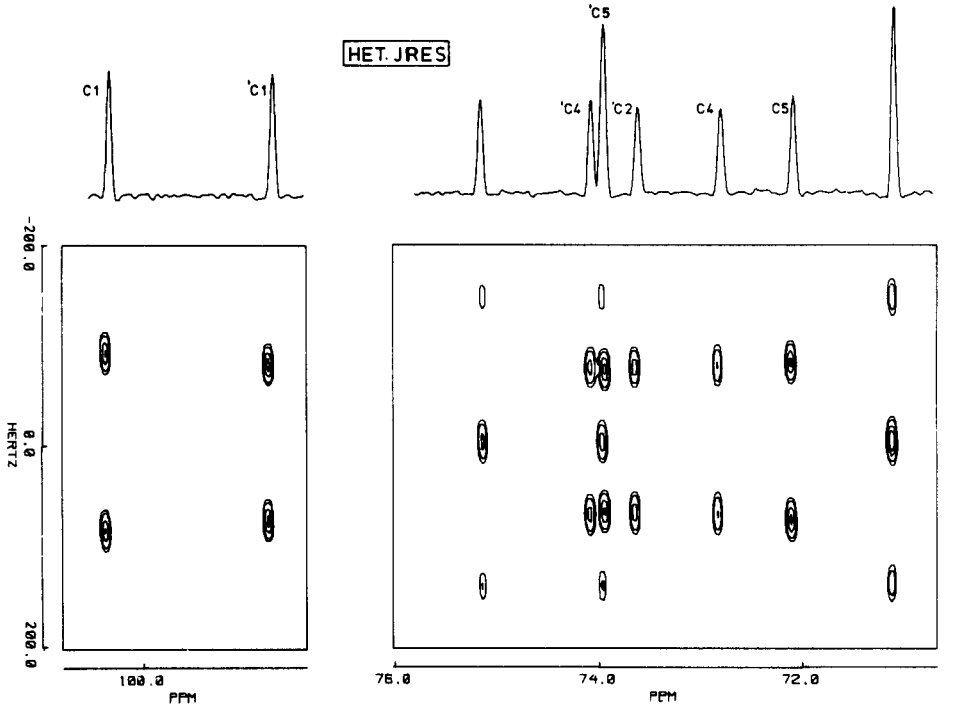


FIG. 2A. Heteronuclear J-resolved NMR spectrum of compound 7.

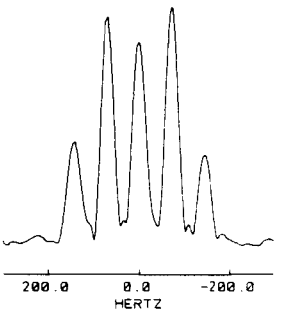


FIG. 2B. Cross-section of the C-5 resonance in 2A revealing a doublet for C-5 and a triplet for -$\underline{C}H_2Ph$.

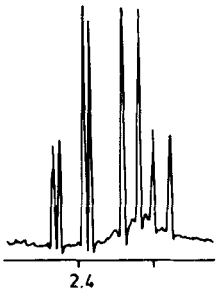


FIG. 3A. ^{1}H NMR spectrum of the $-CH_2CO$ moiety of optical pure 3-(R)-hydroxy-tetradecanoic acid methyl ester in the presence of (+)-2,2,2-trifluoro-1-(9-anthryl) ethanol.

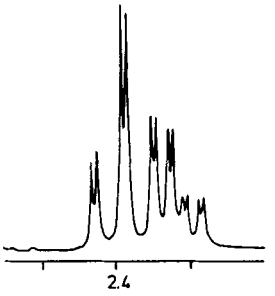


FIG. 3B. ^{1}H NMR spectrum of the $-CH_2CO$ moiety of racemic 3-(R)-hydroxytetra-decanoic acid methyl ester in the presence of the same chiral shift reagens as in 3A.

hydrogen sulfide in pyridine/water[38] to afford **16**. Infrared spectrometry of the di-amino derivative **16** revealed complete disappearance of the characteristic azide absorption bands, thus confirming reduction of both azido functions. *N*-Acylation of **16** with 3-(R)-hydroxytetradecanoic acid[39] **17** in the presence of *N*-ethylmorpholine, 1-hydroxybenzotriazole, and dicyclohexylcarbodiimide gave glycolipid **20** in 88% yield.

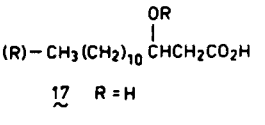
The optical purity of 3-(R)-hydroxytetradecanoic acid **17** was determined by ^{1}H NMR spectroscopy of its methyl ester in

the presence of a chiral shift reagent. A small sample of 17 was treated with diazomethane in dichloromethane. The ^{1}H NMR (300 MHz) spectrum of the α-methylene group of 3-(R)-hydroxy-tetradecanoic acid methyl ester together with the chiral shift reagent[40] (+)-2,2,2-trifluoro-1-(9-anthryl)ethanol is illustrated in FIG.3A. The ^{1}H NMR spectrum of a racemic sample of the corresponding methyl ester is shown in Fig.3B. It can be seen that addition of the shift reagent to the racemate resulted in enantiomeric splitting. Using this technique optical impurities of less than 5% could be detected.

Acylation of **20** with octanoic anhydride in pyridine in the presence of *N,N*-dimethylaminopyridine afforded glycolipid **21** in a yield of 92%.

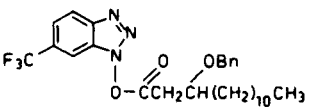
Hydrogenolytic cleavage (Pd/C) of the benzyl groups of **20** and **21** in DMF furnished, Lipid A derivatives **22** and **23** in 89% and 57% yields respectively. The ^{1}H and ^{13}C NMR spectra of **22** and **23** thus obtained were consistent with the proposed structures.

Preparation of Lipid A derivatives 30 and 33. The molecules to be synthesized (i.e. compounds **30** and **33**) contain an ester function at C-3. For this reason it is necessary to replace the acetate esters at C-6 and C-6' by benzyl ethers. Thus, Zemplén deacetylation of key disaccharide **24** afforded **25**. Benzylation of the di-hydroxy derivative **25** with NaH and benzyl bromide in THF in the presence of tetrabutylammonium iodide[41] was straightforward and furnished compound **26** in 80% yield. The introduction of the *O,N*-acyl functions was performed as follows. Selective removal of the allyl protective group followed by reduction of the azido group would give compound **28**. However, isomerization of the allyl ether into a



17 R = H

18 R = Bn



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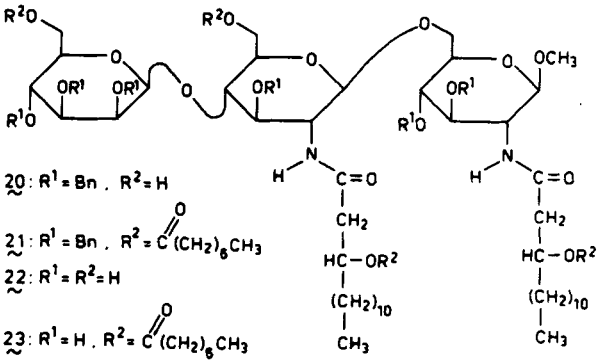
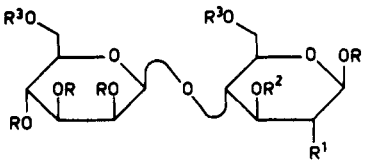


20: R^1 = Bn, R^2 = H

21: R^1 = Bn, R^2 = $C(=O)(CH_2)_6CH_3$

22: R^1 = R^2 = H

23: R^1 = H, R^2 = $C(=O)(CH_2)_6CH_3$



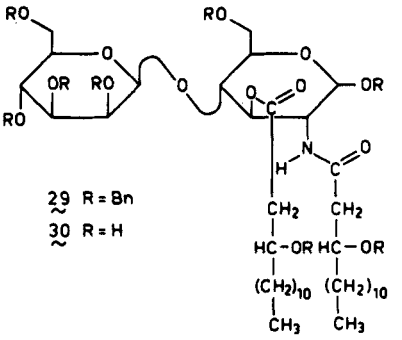
24 R = Bn, R^1 = N_3, R^2 = Allyl, R^3 = Ac

25 R = Bn, R^1 = N_3, R^2 = Allyl, R^3 = H

26 R = R^3 = Bn, R^1 = N_3, R^2 = Allyl

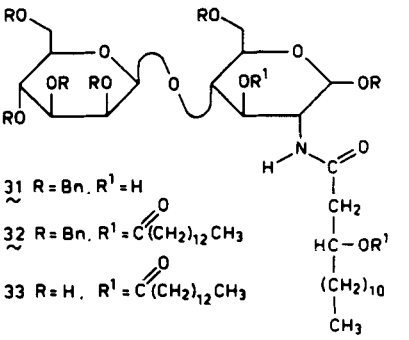
27 R = R^3 = Bn, R^1 = N_3, R^2 = H

28 R = R^3 = Bn, R^1 = NH_2, R^2 = H



29 R = Bn

30 R = H



31 R = Bn, R^1 = H

32 R = Bn, R^1 = $C(=O)(CH_2)_{12}CH_3$

33 R = H, R^1 = $C(=O)(CH_2)_{12}CH_3$

prop-1-enyl ether, using 1,5-cyclooctadiene-*bis*[methyldiphenylphosphine]-iridium hexafluorophosphate[42], failed. Fortunately, treatment of **26** with palladium(II)chloride[43] to remove the allyl group yielded the hydroxy derivative **27**, although in a rather low yield of 46%. After selective reduction of the azido function, to give **28**, the 2-amino and 3-hydroxyl functions were acylated with the activated ester **19**, obtained *in situ* from the reaction of 3-(R)-benzyloxytetradecanoic acid **18**[44] with 1,1'-bis(6-(trifluoromethyl)benzotriazolyl)-oxalate[45] to give fully protected Lipid A derivative **29** in 62% yield. Finally, hydrogenolytic deprotection of **29** afforded Lipid A derivative **30** in 84% yield. The identity of Lipid A derivative **30** was established by ^{1}H, ^{13}C NMR spectroscopy and FAB MS.

Selective introduction of 3-(R)-hydroxytetradecanoic acid on the free 2-amino group of **28** to yield **31** was accomplished using 1-hydroxybenzotriazole and dicyclohexylcarbodiimide. Acylation of **31** with tetradecanoic anhydride in the presence of *N,N*-dimethylaminopyridine gave fully protected Lipid A derivative **32** in 55% yield. The benzyl groups of **32** were removed by catalytic hydrogenation to afford Lipid A derivative **33** in yield of 98%, the identity of which was corroborated by ^{1}H, ^{13}C NMR spectroscopy and FAB MS.

Lipid A derivatives **22**, **23**, **30** and **33** were used to study their biological activities *in vitro* and *in vivo* assays. Preliminary results[46] indicated that compound **23** was active in a lymphocyte transformation test. Compound **23** and **33** exhibited strong immunostimulating (adjuvant) activity in mice as indicated by an increase in serum antibody level against bovine serum albumin.

The above mentioned synthesis of Lipid A derivatives 22, 23, 30 and 33 demonstrates that a combined use of 2-azido-2-deoxy-D-glucopyranosyl units[47] as synthons together with heterogeneous silver silicate, as a promoter for β-glycosilation reactions, can be successfully applied in the synthesis of Lipid A derivatives.

EXPERIMENTAL

General Procedures. Tetrahydrofuran, dioxane and pyridine were dried by heating with CaH_2 under reflux and then distilled. Ether, dichloromethane, chloroform and toluene were distilled from P_2O_5. DMF was stirred with CaH_2 at room temperature and distilled under reduced pressure. Pyridine, DMF and tetrahydrofuran were stored over molecular sieves 4A. Toluene and ether were stored over sodium wire and dichloromethane and chloroform over alumina. Schleicher and Schull DC Fertigfolien F1500 LS254 and Merck-Fertigplatten (Kieselgel 60F 254, 5 x 10 cm) were used for TLC analysis. Compounds were detected by charring with 20% sulfuric acid in methanol, or by spraying with 1% potassium permanganate in 5% aqueous potassium carbonate for compounds containing an alkenic component. Optical rotations were recorded at ambient temperature with a Perkin-Elmer 241 polarimeter. Column chromatography was performed on Kieselgel 60, 70-230 Mesh (Merck). Gel filtration was performed on Sephadex LH20 (Pharmacia). ^{13}C NMR spectra were recorded at 50.1 MHz with a Jeol JNM-FX200 spectrometer. ^{1}H NMR spectra were recorded on a Bruker WM-300 spectrometer equipped with an ASPECT 2000 computer. Chemical shifts are given in ppm (δ) relative to TMS as internal reference, unless otherwise stated.

1,6-Anhydro-2-azido-2-deoxy-3-O-benzyl-4-O-(6-O-acetyl-2,3,4-tri-O-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (6). To a stirred solution of 1,6-anhydro-2-azido-2-deoxy-3-*O*-benzyl-β-D-glucopyranose (8 mmol, 2.22 g) in toluene/dichloromethane (20 mL, 3/1, v/v) containing silver silicate (8 g) and molecular sieves 4A (4 g), was added dropwise a solution of 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl bromide 2 (7 mmol, 3.89 g) in toluene (10 mL) at -50°. Stirring was continued for 3 h, when TLC analysis (CH_2Cl_2/-acetone, 97/3) revealed the formation of one major product. CH_2Cl_2 (150 mL) was added and the mixture stirred 15 min before filtration over celite. The filtrate was concentrated to dryness and the oily residue was applied to a column of Sephadex LH-20 (150 x 3 cm) in 2/1 CH_2Cl_2/MeOH. The combined appropriate fractions were concentrated to dryness and the residual oil was purified by silica gel column chromatography (toluene/acetone, 95/5) yielding 71% of 6-β and 9% of 6-α as colourless oils: $[\alpha]^{20}$ -32° (c 1, $CHCl_3$); R_F 0.57 (toluene:acetone, 5:1); ^1H NMR ($CDCl_3$) δ 1.93 (s, 3H, CH_3CO), 3.27 (s, 1H, H-2), 3.46 (ddd, 1H, H-5', $J_{4',5'}$ 9.6 Hz, $J_{5',6a'}$ 2.1 Hz, $J_{5',6b'}$ 5.7 Hz), 3.52 (dd, 1H, H-3', $J_{2',3'}$ 3.0 Hz, $J_{3',4'}$ 9.4 Hz), 3.74 (c, 1H, H-3), 3.79 (dd, 1H, H-6b, $H_{5,6b}$ 6.0 Hz, $J_{6a,6b}$ 7.3 Hz), 3.91 (c, 1H, H-4), 3.92 (t, 1H, H-4', $J_{3',4'}$=$J_{4',5'}$ 9.5 Hz), 4.04 (d, 1H, H-2', $J_{1',2'}$ 3.0 Hz), 4.16 (dd, 1H, H-6a, $J_{5,6a}$ = 1.1 Hz, $J_{6a,6b}$ 7.3 Hz), 4.23 (dd, 1H, H-6b', $J_{6a'6b'}$ = 11.8 Hz, $J_{5',6b'}$ = 5.7 Hz), 4.47 (dd, 1H, H-6a', $J_{5',6a'}$ = 2.1 Hz, $J_{6a',6b'}$ = 11.8 Hz), 4.49 (d, 1H, CH_2Ph), 4.54 (d, 1H, CH_2Ph), 4.55 (d, 1H, CH_2Ph), 4.66 (d, 1H, CH_2Ph), 4.63 (c, 1H, H-5), 4.69 (s, 1H, H-1'), 4.88 (d, 1H, CH_2Ph), 4.96 (d, 1H, CH_2Ph), 5.01 (d, 1H, CH_2Ph),

5.51 (bs, 1H, H-1), 7.04-7.39 (m, 20H, Ph), ^{13}C NMR ($CDCl_3$) δ 20.80 ($\underline{C}H_3CO$), 59.31 (C-2), 63.37 (C-6'), 64.82 (C-6), 71.24, 72.27, 74.17, 75.21 (4 x CH_2Ph), 72.28 (C-5), 72.95 (C-4), 73.78 (C-2'), 74.04 (C-5'), 74.15 (C-4'), 77.53 (C-3), 81.90 (C-3'), 98.77 (C-1'), 100.48 (C-1), 127.28-128.42 (4x Ph), 137.33, 137.77, 137.89, 138.76 (4x Cquat. Ph), 170.65 (CO).

Anal. Calcd for $C_{42}H_{45}O_{10}N_3$: C, 67.0; H, 6.0. Found: C, 66.9; H, 6.0.

1,6-anhydro-2-azido-2-deoxy-3-O-allyl-4-O-(6-O-acetyl-2,3,4-tri-O-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (7). Compound 7 was obtained from bromide 2 and aglycon 4 similarly to the preparation of 6: yield 66%; $[\alpha]^{20}$ -35° (*c* 1, $CHCl_3$); R_F 0.63 (toluene/acetone, 5/1); ^1H NMR ($CDCl_3$) δ 2.03 (3H, CH_3CO), 3.25 (c, 1H, H-2), 3.46 (ddd, 1H, H-5', $J_{4',5'}$ = 9.6 Hz, $J_{5',6a'}$ = 5.7 Hz, $J_{5',6b'}$ = 2.1 Hz), 3.55 (dd. 1H, H-3', $J_{2',3'}$ = 3.0 Hz, $J_{3',4'}$ = 9.5 Hz), 3.69 (c, 1H, H-3), 3.78 (dd, 1H, H-6b, $J_{5,6b}$ = 6.0 Hz, $J_{6a,6b}$ = 7.3 Hz), 3.82 (ddd, 1H, H-4, $J_{4,5}$ = 1.1 Hz), 3.92 (dd, 1H, H-4', $J_{3',4'}$ = 9.5 Hz, $J_{4',5'}$ = 9.6 Hz), 4.04 (dd, 1H, H-2', $J_{1',2'}$ = 0.3 Hz, $J_{2',3'}$ = 3.0 Hz), 4.10 (dd, 1H, H-6a, $J_{5,6a}$ = 1.2 Hz), 4.23 (dd, 1H, H-6a', $J_{6a',6b'}$ = 11.7 Hz), 4.45 (dd, 1H, H-6b'), 4.64 (c, 1H, H-5), 4.68 (d, 1H, H-1', $J_{1',2'}$ = 0.3 Hz), 5.512 (bs, 1H, H-1), 4.49-5.05 (6H, 3x CH_2Oh), 4.12 (c, OCH_2 allyl), 5.21 (c, 2H, $=CH_2$), 5.92 (m, 1H, CH=), 7.03-7.40 (c, 15H, Ph), ^{13}C NMR ($CDCl_3$) δ 20.79 ($\underline{C}H_3CO$), 59.19 (C-2), 63.31 (C-6'), 64.68 (C-6), 71.13, 117.56, 133.74 (3x C allyl), 72.12 (C-5), 72.80 (C-4), 73.58 (C-2'), 73.93 (C-5'), 74.05 (C-4'), 77.17 (C-3), 81.79 (C-3'), 98.78 (C-1', J_{CH} = 154 Hz), 100.33 (C-1, J_{CH} = 177 Hz), 71.14, 73.96, 75.16 (3x $\underline{C}H_2Ph$), 127.25-128.31 (3x Ph), 137.82, 138.69 (3x Cquat Ph), 170.59 ($CH_3\underline{C}O$).

Anal. Calcd for $C_{38}H_{43}O_{10}N_3$: C, 65.0; H, 6.1. Found: C, 65.1; H, 5.9.

1,6-Di-O-acetyl-3-O-allyl-2-azido-2-deoxy-4-O-(6-O-acetyl-2,3,4-tri-O-benzyl-β-D-mannopyranosyl)-α/β-D-glucopyranose (11). To a stirred solution of 7 (2.1 g, 3.0 mmol) in acetic anydride (58 mL) was added dropwise at 0°C trifluoroacetic acid (1.8 mL). After stirring for 3h at 25°C, TLC analysis (CH_2Cl_2-acetone, 97:3) showed conversion of the starting material into 11. The solution was coevaporated with toluene (3x 100 mL) and the residue was purified by silica gel column chromatography (eluent CH_2Cl_2-acetone, 99:1) to afford 11 (2.4 g, 99%): $[\alpha]_{20}$ -19° (*c* 1, $CHCl_3$) α-acetate; R_F 0.31 (CH_2Cl_2 : acetone, 97:3) α-acetate; ^1H NMR ($CDCl_3$) α-acetate δ 4.42 (bs, 1H, H-1'), 6.16 (d, 1H, H-1, $J_{1,2}$ = 4.0 Hz); ^{13}C NMR ($CDCl_3$) α-acetate δ 20.76, 20.82, 20.91 (3x $COCH_3$), 62.14 (C-6); 63.42 (C-6'), 90.14 (C-1), 101.59 (C-1'), 168.66, 170.38, 170.70 (3x $COCH_3$).

1,6-Di-O-acetyl-4-O-(6-O-acetyl-2,3,4-tri-O-benzyl-β-D-mannopyranosyl)-2-azido-2-deoxy-3-O-benzyl-α/β-D-glucopyranose (8). Compound 8 was obtained in the same way as described for the synthesis of 11, starting from 6. The analytical data of 8 are comparable with those of 11.

6-O-acetyl-4-O-(6-O-acetyl-2,3,4-tri-O-benzyl-β-D-mannopyranosyl)-3-O-allyl-2-azido-2-deoxy-α-D-glucopyranosyl bromide (13). A solution of compound 11 (2.4 g, 2.99 mmol) in dry dioxane (25 mL) was treated for 16h with piperidine (1.42 mL). Dichloromethane (150 mL) was added and the mixture was washed with diluted hydrochloric acid (50 mL), 0.01 N), water (50 mL), aqueous sodium bicarbonate (50 mL, 10%, w/v) and water (50 mL), dried ($MgSO_4$) and concentrated to dryness. The

residual oil was purified by column chromatography (silica gel, eluent CH_2Cl_2/acetone, 9:1) to afford pure 12 (2.1 g, 92%, R_F 0.37 (CH_2Cl_2/acetone, 9:1). To a vigorous stirred solution of 12 (2.1 g, 2.75 mmol) in chloroform (25 mL) and N,N-dimethylformamide (3.7 mL) was added a solution of oxalyl bromide in chloroform (8 mL, 1M) at 0°C. The mixture was stirred for 2 h at 20°C when TLC analysis (CH_2Cl_2/acetone, 95:5) showed the reaction to be complete. The reaction mixture was diluted with ether (150 mL), filtered and washed with cold aqueous sodium bicarbonate (50 mL, 10%, w/v) and cold saturated NaCl solution (50 mL) and dried ($MgSO_4$). Evaporation of the solvent afforded crude 13, which was immediately used for further reaction: yield 1.91 g (84%); $[\alpha]^{20}$ + 39° (c 1, $CHCl_3$); R_F 0.54 (CH_2Cl_2/acetone, 95:5); ^1H NMR ($CDCl_3$) δ 2.03, 2.06 (2x s, 6H, 2x CH_3); 3.41 (ddd, 1H, H-5', $J_{4',5'}$ = 9.6 Hz, $J_{5',6a'}$ = 3.6 Hz), 3.46-3.55 (c, 2H, H-2, H-4), 3.52 (dd, 1H, H-3', $J_{2',3'}$ = 3.0 Hz, $J_{3',4'}$ = 9.2 Hz), 3.77 (d, 1H, H_{6b}, $J_{6a,6b}$ = 9.7 Hz), 3.79 (d, 1H, H-3), 3.87 (d, 1H, H-2'), 3.91 (t, 1H, H-4'), 4.12 (dd, 1H, H-6a, $J_{5,6a}$ = 3.0 Hz); 4.16 (d, 1H, H-5); 4.33 (d, 2H, H-6a', H-6b'); 4.36 (s, 1H, H-1'); 4.53-4.95 (c, 6H, 3x CH_2Ph); 5.12-5.21 (c, 2H,=CH_2); 5.92 (o, 1H,=CH); 6.32 (d, 1H, H-1, $J_{1,2}$ = 3.8 Hz); 7.02-7.39 (c, 15H, 3x Ph).

6-O-Acetyl-4-O-(6-O-acetyl-2,3,4-tri-O-benzyl-β-D-mannopyranosyl)-2-azido-2-deoxy-3-O-benzyl-α-D-glucopyranosyl bromide (10). Compound 9 (1.73 g, 2.13 mmol) was treated as described for the preparation of compound 13: yield 1.85 g (98%); R_F 0.52 (CH_2Cl/acetone, 95:5); ^1H NMR ($CDCl_3$) δ 4.41 (bs, 1H,H-1'), 6.34 (d, 1H, H-1, $J_{1,2}$ = 4.2 Hz).

Methyl-O-(6-O-acetyl-2,3,4-tri-O-benzyl-β-D-mannopyranosyl)-(1-4)-O-(6-O-acetyl-2-azido-2-deoxy-3-O-benzyl-β-D-glucopyranosyl)-(1-6)-2-azido-2-deoxy-3,4-di-O-benzyl-β-D-glucopyranoside (14). A mixture of alcohol 5 (1.88 mmol), silver silicate (2 g) and 4A molecular sieves (1 g) in dichloromethane (5 mL) was stirred for 1 h at 20°C. Then the mixture was cooled to -20°C and a solution of glycosyl bromide 10 (2.0 mmol) in dichloromethane (3 mL) was added dropwise. The mixture was stirred for 3 h at -20°C, when TLC indicated the formation of 14. Dichloromethane (100 mL) was added to the mixture, solids were removed by filtration and the clear filtrate was concentrated to dryness. The residual oil was applied to a column of Sephadex LH-20 (120 x 3 cm^2) eluted with CH_2Cl_2/MeOH, 2:1. The appropriate fractions were collected, concentrated and the residue was chromatographed on Kieselgel 60 (CH_2Cl_2/acetone, 97:3 - 95:5) to give 14 as a colourless oil: yield 1.35 g (60%); R_F 0.46 (CH_2Cl_2/acetone, 95:5); $[\alpha]^{20}$ -25° (*c* 1, $CHCl_3$); ^1H NMR (360 MHz) ($CHCl_3$) δ 2.03, 2.05 (2x s, 6H, 2x CH_3); 3.39-3.58 (c, 8H, H-2, H-3, H-4, H-2', H-3', H-5', H-3'', H-5''), 3.61 (ddd, 1H, H-5, $J_{4,5}$ = 9.5 Hz, $J_{5,6b}$ = 2.1 Hz), 3.65 (s, 3H, OCH_3), 3.71 (dd, 1H, H-6a, $J_{5,6a}$ = 5.7 Hz, $J_{6a,6b}$ = 11.4 Hz), 3.86 (dd, 1H, H-4', $J_{3',4'}$ = 10 Hz, $J_{4',5'}$ = 8.1 Hz), 3.92 (t, 1H, H-4'', $J_{3'',4''}$ = $J_{4'',5''}$ = 9.5 Hz), 3.93 (bd, 1H, H-2'', $J_{2'',3''}$ = 2.8 Hz), 4.18-4.36 (c, 5H, H-6b, H-6a', H-6b', H-6a'', H-6b''), 4.27 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.39 (d, 1H, H-1', $J_{1,2}$ = 7.9 Hz), 4.51 (bs, 1H, H-1''), 4.59-5.14 (c, 12H, 6x CH_2Ph), 7.01-7.42 (c, 30H, Ph); ^{13}C NMR ($CDCl_3$) δ 20.47, 20.56 (2x CH_3), 56.91 (OCH_3), 62.17 (C-6'), 63.10 (C-6''), 65.68 (C-2'), 66.02 (C-2), 68.50 (C-6), 72.62 (C-5'), 73.50

(C-5''), 73.73 (C-4''), 74.12 (C-5), 74.49 (C-2''), 76.82 (C-4'), 77.61 (C-4), 80.12 (C-3'), 82.08 (C-3''), 82.81 (C-3), 100.68 (C-1), 102.17 (C-1'), 102.67 (C-1''), 71.51, 73.96, 74.24, 74.84, 75.22 (6x CH_2Ph); 126.61-128.19 (6x Ph), 137.47-138.32 (6x Cquat Ph), 170.35 (2x $\underline{C}OCH_3$).

Anal. Calcd for $C_{65}H_{72}O_{16}N_6$: C, 65.4; H, 6.0. Found: C, 65.2; H, 6.0.

Methyl-O-(2,3,4-tri-O-benzyl-β-D-mannopyranosyl-(1-4)-O-(2-azido-2-deoxy-3-O-benzyl-β-D-glucopyranosyl)-(1-6)-2-azido-2-deoxy-3,4-di-O-benzyl-β-D-glucopyranoside (15). A catalytic amount of potassium *tert*-butoxide was added to a solution of trisaccharide 14 (0.97 g, 0.81 mmol) in a mixture of methanol (2.5 mL) and dioxane (3.5 mL). After 20 min Dowex 50 H^+) resin (1 g) was added. The resin was filtered off, washed and the filtrate was concentrated to give 15: yield 98%; R_F 0.37 (CH_2Cl_2/ acetone, 9:1); $[\alpha]^{20}$ -9° (*c* 1, $CHCl_3$).

Methyl-O-(2,3,4-tri-O-benzyl-β-D-mannopyranosyl)-(1-4)-O-(3-O-benzyl-2-deoxy-2-[(R)-3-hydroxytetradecanoylamino]-β-D-glucopyrano-syl)-(1-6)-3,4-di-O-benzyl-2-deoxy-2-[(R)-3-hydroxytetradecanoyl-amino]-β-D-glucopyranoside (20). Compound 15 (0.65 g, 0.59 mmol) was dissolved in pyridine/water (20 ml, 4:1, v/v) and a gentle stream of H_2S was bubbled through the mixture for 20 h. TLC analysis indicated conversion of the starting material into 16. The reaction mixture was concentrated and the residue was purified by silica gel column chromatography (eluent CH_2Cl_2/MeOH/Et_3N, 90:10:0.05-80:20:0.05) to yield the pure diamino-trisaccharide 16 in 92%. R_F 0.42 (CH_2Cl_2/MeOH, 9:1). To a solution of 16 (0.57 g, 0.54 mmol), 1-hydroxy-benzotriazole (1.56 mmol), (R)-3-hydroxytetradecanoic acid 17 (0.38 g, 156 mmol) and tri-

ethylamine (5 mmol) in dioxane (8 mL) dicyclohexylcarbodiimide (1.56 mmol) was added and the mixture was stirred at 20°C for 2 h. The solvent was evaporated, the residue was taken up in CH_2Cl_2 (50 mL) and successively washed with aqueous sodium bicarbonate (25 ml, 10%, w/v) and water (25 mL), dried ($MgSO_4$) and concentrated to dryness. The residue was applied to a column of silica gel and eluted with CH_2Cl_2/MeOH (93:7 - 9:1) to give glycolipid **20** (0.72 g, 88%): mp 134-135°; $[\alpha]^{20}$ -26° (c 1, $CHCl_3$); ^{13}C NMR δ 14.29 (2x CH_3); 37.37, 37.62 (2x α-CH_2 myristoyl); 55.79, 55.81 (C-2, C-2'), 57.13 (OCH_3), 61.39 (C-6'), 62.45 (C-6''); 68.40 (C-6); 68.98, 69.11 (2x α-CH9OH) myristoyl), 80.54 (C-3), 83.04, 83.11 (C-3', C-3''), 101.03 (C-1''), 101.44 (C-1'), 102.60 (C-1), 173.84, 173.86 (2x CO).

Methyl-O-(2,3,4-tri-O-benzyl-6-O-octanoyl-β-D-manno-pyranosyl)-(1-4)-O-(3-O-benzyl-2-deoxy-2-[(R)-3-octanoyloxy-tetradecanoylamino]-6-O-octanoyl-β-D-glucopyranosyl)-(1-6)-3,4-di-O-benzyl-2-deoxy-2-[(R)-3-octanoyloxytetradecanoyl-amino]-β-D-glucopyranoside (21). To a solution of compound **20** (0.25 g, 0.165 mmol) in a mixture of CH_2Cl_2 and pyridine (4 mL, 7:1, v/v) were added octanoic anhydride (0.27 g, 1 mmol) and a catalytic amount of DMAP. After 16 h at 20°C, TLC analysis (CH_2Cl_2/acetone, 9:1) indicated complete conversion of starting material **20** (R_F 0.45) into the desired product **21** (R_F 0.80). CH_2Cl_2 (50 mL) was added and the mixture was washed with aqueous sodium bicarbonate (25 mL, 10%, w/v) and water (25 mL), dried ($MgSO_4$) and concentrated. The residue was purified on a column of silica gel eluted with CH_2Cl_2/acetone, 95:5 - 9:1, to give compound **21** as a waxy solid: yield 0.319 (92%); R_F 0.8 (CH_2CL_2/acetone, 9:1); $[\alpha]^{20}$

-6° (c 0.5, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 14.05, 14.07 (6x CH_3); 22.54-31.96 (40x $-CH_2-$, fatty acids), 33.91-34.63 (4x CH_2CO), 41.19-41.92 (2x CH_2CONH), 56.12, 56.33 (C-2, C-2'); 56.48 (OCH_3); 63.13, 63.18 (C-6', C-6''); 67.49 (C-6); 70.91, 71.12 (2x CH-OCO); 71.91, 73.21, 73.86, 73.97, 74.02, 74.09, 74.12, 74.36, 75.12, 77.09, 78.22, 80.51 (C-2'', C-3, C-3', C-4, C-4', C-4'', C-5, C-5', C-5'', 6x CH_2Ph); 82.46 (C-3''); 99.86, 100.74, 101.45 (C-1'', C-1', C-1); 126.70-128.21 (6x Ph); 137.36-138.31 (6x Cquat Ph); 169.31, 169.46 (2x CONH); 173.21, 173.36 (4x CO); ^1H NMR ($CDCl_3$) δ 0.81-0.91 (c, 18 H, 6x CH_3), 1.13-1.65 (c, 80 H, 2x $(CH_2)_{10}$, 4x $(CH_2)_5$; 2.11-2.36 (c, 12 H, 6x CH_2CO), 5.05 (c, 2 H, 2x CHOCO); 5.96, 6.21 (2x d, 2x NH, $J_{2,NH}$ = 9Hz); 7.19-7.42 (c, 30 H, 6x Ph).

Methyl-O-(β-D-mannopyranosyl)-(1-4)-O-(2-deoxy-2-[(R)-3-hydroxytetradecanoylamino]-β-D-glucopyranosyl)-(1-6)-2-deoxy-2-[(R)-3-hydroxytetradecanoylamino]-β-D-glucopyranoside (22). A solution of compound **20** (0.28 g, 0.19 mmol) in ethanol/*N*,*N*-dimethylformamide/ acetic acid (20 mL, 14:5:1) was hydrogenated at 20°C and atmospheric pressure in the presence of 10% palladium/charcoal catalyst (200 mg). After 16 h, the catalyst was removed by filtration and the filtrate was concentrated. Purification of the residue on a column of Sephadex LH-20 (45 x 3 cm) in DMF afforded 89% of Lipid A derivative **22**. $[\alpha]^{20}$ -25° (c 1, DMF); R_F 0.15 ($CHCl_3$/acetone/MeOH/AcOH/H_2O, 50:20:10:10:5); mp 218-221°C (dec.); ^{13}C NMR (DMF-d7) δ 14.28 (2x CH_3); 23.11, 26.20, 26.25, 32.40, 37.60, 37.85 (2x $(CH_2)_{10}$); 44.31, 44.39 (2x CH_2CO); 56.14, 56.48 (C2, C-2'); 56.51 (OCH_3); 68.81, 68.89 (2x CHOH); 61.54, 62.52, 68.35, 68.89, 71.89, 73.46, 75.05, 75.91, 76.24, 76.52, 78.51, 81.31 (C-3-C-6, C-3'-C-6', C-2''-C-6''); 102.83 (C-1); 102.73 (C-1'); 102.01 (C-1''); 172.38; 172.76 (2x CONH).

Anal. Calcd for $C_{47}H_{90}O_{20}N_2$: C, 56.3; H, 9.0. Found: C, 56.4; H, 8.8.

Methyl-O-(6-O-octanoyl-β-D-mannopyranosyl)-(1-4)-O-(2-deoxy-2-[(R)-3-octanoyloxytetradecanoylamino]-6-O-octanoyl-β-D-glucopyranosyl)-(1-6)-2-deoxy-2-[(R)-3-octanoyloxytetradecanoylamino]-β-D-glucopyranoside (23). Compound 21 (0.22 g, 0.147 mmol) was dissolved in 2-propanol/N,N-dimethylformamide/acetic acid, 14:2:1 and hydrogenated in the presence of 10% palladium on charcoal (200 mg) for 16 h. The mixture was filtered, the filtrate was concentrated and the residue applied to a column of Sephadex LH-20 (45 x 3 cm) in 1:1 CH_2Cl_2 to give pure Lipid A derivative 23 (0.12 g, 57%). $[\alpha]^{20}$ -13° (c 1, CH_2Cl_2/MeOH, 1:1); R_F 0.51 (CH_2Cl_2/MeOH, 85.15); mp 190-192° (dec); ^{13}C NMR ($CDCl_3/CD_3OD$, 1:1) δ 14.09 (6x CH_3), 22.71-32.04 (40x (CH_2)); 34,01, 34.26, 34.63, 34.70 (4x CH_2CO); 41.40, 41.83 (2x CH_2CONH); 56.04, 56.22 (C-2, C-2'); 56.53 (OCH_3), 62.86 (C-6'), 63.67 (C-6''), 67.37 (C-4''), 68.63 (C-6), 71.53, 71.64 (2x CHOCO); 70.70, 71.02, 72.14, 72.57, 73.71, 74.66, 74.82, 74.99, 81.03 (C-3, C-4, C-5, C-3', C-4', C-5', C-2'', C-3'', C-5''); 100.95 (C-1''); 101.09 (C-1'), 101.76 (C-1); 171.36, 171.78 (2x CONH); 174.25, 174.30, 174.36, 174.50 (4x CO).

Anal. Calcd for $C_{79}H_{146}O_{24}N_2$: C, 62.9; H, 9.7. Found: C, 63.0; H, 9.9.

Benzyl 6-O-acetyl-4-O-(6-O-acetyl-2,3,4-tri-O-benzyl-β-D-mannopyranosyl)-3-O-allyl-2-azido-2-deoxy-β-D-glucopyranose (24). A solution of bromide 13 (2.1 g, 2.54 mmol) in toluene (7.5 mL) was added slowly at -65°C to a solution of benzyl alcohol (3.1 mL) in toluene (30 mL) previously stirred for 2 h with silver silicate (3.45 g) and molecular sieves (1.7 g,

4 A) and the mixture was then vigorously stirred for 2 h at o°C. The reaction mixture was diluted with CH_2Cl_2 (150 mL), filtered and concentrated. The residue was purified on a silica gel column (CH_2Cl_2/acetone, 99:1 - 95:5) to give 24 (1.13 g, 52%): $[\alpha]^{20}$ -18° (*c* 1, $CHCl_3$); R_F 0.59 (CH_2Cl_2/acetone, 95:5); ^1H NMR ($CDCl_3$) δ 3.27 (dd, 1H, H-3, $J_{2,3}$ = 9.1 Hz, $J_{3,4}$ = 10 Hz), 3.36 (bd, 1H, H-5, $J_{4,5}$ = 8.0 Hz), 3.37 (d, 1H, H-2), 3.43 (dd, 1H, H-5', $J_{4,5}$ = 9.6 Hz, $J_{5,6a}$ = 2.7 Hz), 3.51 (dd, 1H, H-3', $J_{2',3'}$ = 2.8 Hz, J3',4' = 9.3 Hz), 3.67 (dd, 1H, H-4), 3.85 (d, 1H, H-2'), 3.88 (t, 1H, H-4', J = 9.5 Hz, 4.15 (dd, 1H, H-6a, $J_{6a,6b}$ = 12 Hz, $J_{5,6a}$ = 4.5 Hz), 4.25 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 4.27 (d, 1H, H-6b), 4.28 (s, 1H, H-6b'), 4.31 (d, 1H, H-6a), 4.43 (bs, 1H, H-1'); 5.08, 5.19, 5.92 (-CH=CH_2); 4.54-4.96 (8H, 4x CH_2Ph); 7.02-7.40 (20H, 4x Ph).

Benzyl 3-O-allyl-2-azido-2-deoxy-6-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (26). To a solution of **24** (1.13 g, 1.32 mmol) in 1:1 methanol/dioxane (16 mL) potassium *tert*-butoxide (50 mg) was added and the mixture was stirred for 30 min, neutralized with Dowex 50 (H^+) resin, filtered and concentrated to give **25** 100%, $[\alpha]^{20}$ -37° (*c* 1, $CHCl_3$). To a stirred solution of crude **25** (2.1 mL) was added benzyl bromide (6.3 mmol) and tetrabutylammonium iodide (0.7 mmol). After 10 min, sodium hydride (6.3 mmol) was added and the mixture was stirred for 3.5 h at 50°C. To the cooled solution was added aq. ammonia (25%, 2 mL), and the separated water layer was extracted with CH_2Cl_2 (2 x 5 mL). The combined organic layers were neutralized at 0°C with aq. HCl. The organic phase was washed with water (2 x 10 mL), dried with $MgSO_4$, and concentrated. The

residue was applied to a column of silica gel (eluent CH_2Cl_2/acetone, 100 - 93:7) to give **26** as a colourless oil in 80% yield: $[\alpha]^{20}$ -34° (c 1, $CHCl_3$); R_F 0.55 (CH_2Cl_2/acetone, 97:3); ^1H NMR ($CDCl_3$) δ 4.26 (d, 1H, H-1, $J_{1,2}$ 8.1 Hz), 4.48 (d, 1H, H-1', $J_{1',2'}$ 2.9 Hz); ^{13}C NMR ($CDCl_3$) δ 65.82 (C-2), 68.62 (C-6), 69.53 (C-6'); 74.08, 74.78, 75.07, 75.98, 77.64, 81.06, 82.61 (C-3 - C-5, C-2' - C-5'); 100.42 (C-1), 101.12 (C-1'); 69.47, 116.48, 135.40 (Allyl).

Benzyl 2-azido-2-deoxy-6-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (27). To a solution of compound **26** (0.45 g, 0.47 mmol) in acetone/acetic acid/H_2O (3 mL, 1:1:1) sodium acetate (180 mg) and palladium (II) chloride (105 mg) were added and the mixture was stirred for 24 h at 20°C. The reaction mixture was filtrated, diluted with CH_2Cl_2 (25 mL), washed with water (10 mL), aqueous sodium bicarbonate (10 mL) and water (10 mL), dried ($MgSO_4$) and concentrated. The residual oil was applied to a column of silica gel (eluent CH_2Cl_2/acetone, 99:1 - 97:3) to afford compound **27** as an oil (0.195 g, 46%): $[\alpha]^{20}$ -25° (c 1, $CHCl_3$); R_F 0.48 (CH_2Cl_2/acetone, 97:3); ^{13}C NMR ($CDCl_3$) δ 65.55 (C-2); 68.36, 69.15 (C-6, C-6'); 70.96, 72.18, 73.50, 73.67, 74.23, 75.22 (6x CH_2Ph); 73.93, 74.11, 74.34, 74.63, 74.75, 81.58, 82.57 (C-3 - C-5, C-2' - C-5'); 100.42 (C-1); 101.76 (C-1'); 126.29 - 138.40 (6x Ph).

Benzyl 2-amino-2-deoxy-6-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-mannopyranosyl)-β-D-glucopyranose (28). A solution of compound **27** (0.195 g, 0.21 mmol) in pyridine/triethylamine/H_2O (6 mL, 4:1:1) was treated with hydrogen sulfide for 2.5 h. The green solution was concentrated, coevaporated with

toluene (20 mL), diluted with CH_2Cl_2 and washed with diluted sodium hydroxide (10 mL, 0.01 N) and water (10 mL) dried ($MgSO_4$) and concentrated to give compound **28** (0.189 g, 100%). Infrared spectroscopy of a neat sample of **28** confirmed complete reduction of the azido function (no N_3 stretch at 2110 cm^{-1}).

Benzyl 4-O-(2,3,4,6-tetra-O-benzyl-β-D-mannopyranosyl)-6-O-benzyl-3-O-[(R)-3-benzyloxytetradecanoyl]-2-deoxy-2-[(R)-3-benzyloxytetradecanoylamino]-β-D-glucopyranose (29). To a suspension of 1,1'-bis[6-(trifluoromethyl)benzotriazolyl]oxalate (129 mg, 0.28 mmol) and (R)-3-benzyloxytetradecanoic acid (92 mg, 0.28 mmol) in acetonitrile (3 mL) was added 4-dimethylaminopyridine (DMAP, 37 mg, 0.3 mmol). After the reaction mixture was stirred for 1 h at 20°C, a solution of compound **28** (97 mg, 0.11 mmol) and DMAP (37 mg) in acetonitrile (2 ml) was added and stirring was continued for an additional 24 h. The reaction mixture was diluted with CH_2Cl_2 (25 mL), washed with aqueous sodium bicarbonate (10 mL, 10% w/v) and water (10 mL), dried ($MgSO_4$) and concentrated. The crude product was purified by silica gel column chromatography (eluent CH_2Cl_2/acetone, 98:2 - 90:10) and Sephadex LH-20 column chromatography (CH_2Cl_2-MeOH, 2:1) to afford **29** as a waxy solid: yield 70 mg (42%); $[\alpha]^{20}$ -26 (*c* 1, $CHCl_3$); R_F 0.45 (CH_2Cl_2/acetone, 97:3); ^1H NMR ($CDCl_3$) δ 4.42 (d, 1H, H-1, $J_{1,2}$ = 8.4 Hz), 4.46 (bs, 1H, H-1'), 5.22 (dd, 1H, H-3, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 9.2 Hz), 6.02 (d, 1H, NH, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ 100.16 (C-1), 100.62 (C-1'); 171.23, 171.73 (2x CONH).

2-Deoxy-2-[(R)-3-hydroxytetradecanoylamino]-3-O-[(R)-3-hydroxytetradecanoyl]-4-O-(β-D-mannopyranosyl)-α/β-D-gluco-

pyranose (30). A solution of compound **29** (50 mg, 0.033 mmol) in *N,N*-dimethylformamide (6 mL) was treated with hydrogen in the presence of 10% palladium on charcoal (70 mg) for 48 h. The catalyst was removed by filtration and the solution was concentrated. The residual solid was resolved in *t*-butanol/water (5 mL, 4:1) and lyophilized to give Lipid A derivative **30** as a fluffy solid (21 mg, 80%): R_F (α/β) 0.22/0.18 ($CHCl_3$/acetone/MeOH/AcOH/H_2O, 50:20:10:10:5); FAB-MS for $C_{40}H_{75}NO_{14}$: FAB(+) glycerol: m/z $[M+H]^+$ 794, FAB(-)glycerol: m/z $[M]^{-\cdot}$ 793; ^1H NMR ($CDCl_3/CD_3OD$, 1:1) δ 4.59 (s, 1H, H-1'), 5.13 (d, 1H, H-1α, $J_{1,2}$ = 3.6 Hz), 5.34 (dd, 1H, H-3α, $J_{2,3}$ = 10.9 Hz, $J_{3,4}$ = 9.0 Hz), ^{13}C NMR ($CDCl_3/CD_3OD$, 1:1) δ 14.20 (2x CH_3); 22.90, 25.87, 25.96, 29.58, 29.90, 32.15, 36.97, 37.32 (2x-$(CH_2)_{10}$); 42.60, 43.42 ($CH_2C(O)O$, CH_2CONH); 52.21 (C-2); 71.06, 61.21 (C-6, C-6'); 66.58 (C-4'); 68.48, 68.80 (2x β-C myristoyl); 70.93, 71.28, 71.57, 74.11, 75.25, 76.80 (C-3-C-5, C-2', C-3', C-5'); 91.40 (C-1α), 99.89 (C-1'); 173.36, 173.42 (2x CO).

Anal. Calcd for $C_{40}H_{75}O_{14}N$: C, 60.5; H, 9.5. Found: C, 60.7; H, 9.6.

Benzyl 6-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-mannopyranosyl)-2-deoxy-2-[(R)-3-hydroxytetradecanoylamino]-β-D-glucopyranose (31). Compound **28** (189 mg, 0.21 mmol) was acylated with (R)-3-hydroxy tetradecanoic acid **17** (72 mg, 0.30 mmol) as described in the synthesis of compound **20**. The crude product was purified by silica gel column chromatography (eluent CH_2Cl_2/MeOH, 97:3 - 95:5) to give compound **31** as a syrup (214 mg, 90%); $[\alpha]^{20}$ -26 (*c* 1, $CHCl_3$); R_F 0.20 (CH_2Cl_2/MeOH, 97:3); ^{13}C NMR ($CDCl_3$) δ 54.60 (C-2), 100.80 (C-1), 101,21 (C-1').

Benzyl 6-O-benzyl-4-O-(2,3,4,5-tetra-O-benzyl-β-D-mannopyranosyl)-2-deoxy-2-[(R)-3-tetradecanoyloxytetradecanoylamino]-3-O-tetradecanoyl-β-D-glucopyranose (32). Compound **31** (214 mg, 0.193 mmol) was treated with myristic anhydride (2.63 mg, 0.60 mmol) analogous to the synthesis of compound **21**: yield 163 mg (55%); $[\alpha]^{20}$ -22° (*c* 1, $CHCl_3$); R_F 0.28 (CH_2Cl_2/acetone, 97:3); ^1H NMR ($CDCl_3$) δ 0.88 (C, 9H, 3x CH_3), 1.13 - 1.56 (c, 64H, $(CH_2)_{10}$, 2x $(CH_2)_{11}$), 2.13 - 2.47 (c, 6H, 3x CH_2CO); 3.30 (c, 1H, H-5'), 3.35 (dd, 1H, H-3', $J_{2',3'}$ = 3.4 Hz, $J_{3',4'}$ = 9.5 Hz), 3.56 (c, 1H, H-5); 3.60, 3.79 (c, 5H, H-6a, H-6b, H-2', H-6a', H-6b'), 3.87 (t, 1H, H-4', J = 9.5 Hz), 3.98 (t, 1H, H-4, J = 9.2 Hz), 4.12 (q, 1H, H-2, $J_{1,2}$ = $J_{2,3}$ = $J_{2,NH}$ = 9.2 Hz); 4.39 (s, 1H, H-1'), 4.52 (d, 1H, H-1, $J_{1,2}$ = 9.2 Hz); 5.06 (q, 1H, CH_2CHOCO), 5.16 (t, 1H, H-3), 5.84 (cl, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 14.08 (CH_3); 22.66, 24.64, 24.94, 25.23, 29.14, 29.32, 29.41, 29.64, 31.89 (CH_2 fatty acid residues); 33.87, 34.40, 41.64 (3x CH_2CO); 53.44 (C-2); 68.74, 69.41 (C-6, C-6'); 70.05, 71.39, 73.23, 73.47, 73.79, 74.96 (6x CH_2Ph); 71.04 (CH_2CHOCO); 71.69 (C-3); 74.43 (C-4, C-2'); 74.61 (C-4'); 75.01 (C-5); 75.57 (C-5'); 82.20 (C-3'); 100.01 (C-1); 100.68 (C-1'); 169.36 (CONH); 173.48, 173.97 (2x CO).

2-Deoxy-2-[(R)-3-tetradecanoyloxytetradecanoylamino]-4-O-(β-D-mannopyranosyl)-3-O-tetradecanoyl-α/β-D-glucopyranose (33). Compound **32** (163 mg, 0.11 mmol) was treated with hydrogen as described for the synthesis of compound **30**. Lyophylization of the crude product afforded Lipid A derivative as a white fluffy solid. Yield 115 mg (100%). R_F 0.32 ($CHCl_3$/acetone/MeOH/AcOH/H_2O, 50:20:10:10:5): ^{13}C NMR ($CDCl_3/CD_3OD$, 2:1) δ 14.31 (3x CH_3); 23.13, 25.35, 25.55,

25.79, 29.67, 29.85, 30.13, 32.34 (CH_2 fatty acid residues); 34.81, 34.96 (2x CH_2CO); 41.47 (CH_2CONH), 52.85 (C-2); 61.59 (C-6); 62.40 (C-6'); 67.80 (C-4'); 71.19 (C-5); 71.51 (C-3, C-2', CHOCO); 74.40 (C-3'); 75.95 (C-4); 77.17 (C-5'); 91.72 (C-1); 99.93 (C-1'); 171.29 (CONH); 174.38, 175.46 (2x CO).

Anal. Calcd for $C_{54}H_{111}O_{14}N$: C, 65.0; H, 11.1. Found: C, 65.1; H, 11.0.

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