(m, 13 H), 2.76–2.88 (m, 3 H), 4.70 (s, 1 H), 6.29, 6.31, 6.49, 6.51 (AB q, BrCH=CH), 6.56 (d, 2.5 Hz, 1 H, C(1)H); R_f = 0.32 (hexane–ethyl acetate 2:1). Anal. ($C_{20}H_{25}O_4Br \cdot 2.5H_2O$) C, H.

Conditions for the Preparation of 6c/8c. To a solution of 3/4 (0.2 mmol) in CCl_4 (10 mL), stirred at −18 °C, was added dropwise a solution of I_2 in CCl_4 (50 mg in 5 mL). The reaction was warmed to ambient temperature and the solvent was removed by evaporation. The reaction was worked up as previously described. Purification by column chromatography gave pure 6c/8c (95%/96%).

6c: mp 112–115 °C (lit.[14] mp 113–115 °C).

8c: mp 124–126 °C (lit.[21] mp 123–125 °C).

Competitive Receptor Binding Assay. All cytosol for the estrogen receptor were prepared and stored in TEA buffer (0.01 M Tris·HCl, 0.0015 M EDTA, 0.02% sodium azide, pH 7.4 at 25 °C). Rat uterine cytosol was prepared from Holtman rats (21–25-day-old females) and stored in liquid nitrogen. The uterine cytosol was prepared and stored as previously described by Katzenellenbogen et al.[22] The competitive receptor binding assays were performed as previously described[22,23] and the results tabulated as relative binding affinities (RBA) relative to estradiol (RBA = 100).

Acknowledgment. This research has been supported in part by Public Health Service Grant 5-R01-CA-41399. The authors appreciate the assistance of Prof. J. A. Katzenellenbogen, supported by PHS Grant PHS-5-R01-CA-25836, for the receptor binding assays and his critical review of the manuscript.

Registry No. 1, 57-63-6; 2, 5779-47-5; 3, 134970-24-4; 4, 134970-25-5; 6a, 134970-26-6; 6b, 103924-36-3; 6c, 91085-44-8; 8a, 103924-22-7; 8c, 104011-19-0.

C-Glycosidic Analogues of Lipid A and Lipid X: Synthesis and Biological Activities

Hermann Vyplel,* Dieter Scholz, Ingolf Macher, Karl Schindlmaier, and Eberhard Schütze

Sandoz Forschungsinstitut, Brunnerstrasse 59, A-1235 Vienna, Austria. *Received December 26, 1990*

The synthesis of a series of novel analogues of lipid A, the lipophilic terminal of lipopolysaccharides (LPS), and lipid X, the reducing monosaccharide unit in lipid A, is reported. In these compounds, the native 1-*O*-phosphate group has been replaced by a "bioisosteric" CH_2COOH substituent. The new N,O-acylated monosaccharide *C*-glycosides were obtained by Wittig reaction of suitably protected glucosamine derivatives. These lipid X analogues were recognized as substrates by the enzyme lipid A synthase and could be coupled with UDP-lipid X to afford the corresponding disaccharide analogues of the lipid A precursor on preparative scale. All compounds were characterized by NMR, MS, and elemental analysis, and were tested for their ability to enhance nonspecific resistance to infection in mice and also for endotoxicity. The results clearly show that the new compounds express biological activities similar to those of their O-phosphorylated natural counterparts. Furthermore, these compounds exhibit a better therapeutic index in mouse models than the standard LPS obtained from *Salmonella abortus equi*.

Introduction

Lipopolysaccharides (LPS), also called endotoxins, are constituents of the cell walls of Gram-negative bacteria. They cause a wide array of pathophysiological effects and are extremely potent immunostimulants.[1-9] Structurally, they consist of an essentially linear polysaccharide chain anchored to the cell wall by a lipophilic disaccharide, lipid A (1), which is a β-(1→6)-glucosamine disaccharide acylated with several fatty acids—mostly 3-hydroxy myristic acid—and bearing two phosphate groups in positions 1 and 4′ (see Chart I). While the composition of the polysaccharide chains of LPS varies widely among enterobacterial strains, their lipid A's differ only slightly. Lipid A is responsible for most of the immunopharmacological activities of LPS, including the induction of endotoxic shock.[9] Due to their toxicity, neither LPS, nor lipid A have found clinical applications.

Numerous compounds, including biosynthetic lipid A precursors and analogues related to lipid A partial structures have been isolated or synthesized with the aim of separating unwanted endotoxic properties from potentially beneficial immunostimulatory effects.[10-21] Compounds

(1) Galanos, C.; Lüderitz, O.; Rietschel, E. T.; Westphal, O.; Brade, H.; Brade, L.; Freudenberg, M.; Schade, U.; Imoto, M.; Yoshimura, H.; Kusumoto, S.; Shiba, T. *Eur. J. Biochem.* **1985**, *148*, 1.

(2) Homma, J. Y.; Matsuura, M.; Kanegasaki, S.; Kawakubo, Y.; Kojima, Y.; Shibukawa, N.; Kumazawa, Y.; Yamamoto, A.; Tanamoto, K.; Yasuda, T.; Imoto, M.; Yoshimura, H.; Kusomoto, S.; Shiba, T. *J. Biochem.* **1985**, *98*, 395.

(3) Kotani, S.; Takada, H.; Tsujimoto, M.; Ogawa, T.; Harada, K.; Mori, Y.; Kawasaki, A.; Tanaka, A.; Nagao, S.; Tanaka, S.; Shiba, T.; Kusumoto, S.; Imoto, M.; Yoshimura, H.; Yamamoto, M.; Shimamoto, T. *Infect. Immun.* **1984**, *45*, 293.

(4) Kotani, S.; Takada, H.; Tsujimoto, M.; Ogawa, T.; Takahashi, I.; Ikeda, T.; Otsuka, K.; Shimauchi, H.; Mashimo, J.; Nagao, S.; Tanaka, A.; Harada, K.; Nagaki, K.; Kitamura, H.; Shiba, T.; Kusumoto, S.; Imoto, M.; Yoshimura, H. *Infect. Immun.* **1985**, *49*, 225.

(5) Kusumoro, S.; Yamamoto, M.; Shiba, T. *Tetrahedron Lett.* **1984**, *25*, 3727.

(6) Galanos, C.; Rietschel, E. T.; Lüderitz, O.; Westphal, O.; Kim, Y. B.; Watson, D. W. *Eur. J. Biochem.* **1975**, *54*, 603.

(7) Levin, J.; Poore, T. E.; Zauber, N. P.; Oser, R. S. *N. Engl. J. Med.* **1970**, *283*, 1313.

(8) Galanos, C.; Lüderitz, O.; Rietschel, E. T.; Westphal, O. In *International Review of Biochemistry, Biochemistry of Lipids II*; Goodwin, T. W., Ed.; University Park Press: Baltimore, MD 1977; Vol. 14, pp 239–335.

(9) Takahashi, I.; Kotani, S.; Takada, H.; Tsujimoto, M.; Ogawa, T.; Shiba, T.; Kusumoto, S.; Yamamoto, M.; Hasegawa, A.; Kiso, M.; Nishijima, M.; Harada, K.; Tanaka, S.; Okumura, H.; Tamura, T. *Infect. Immun.* **1987**, *65*, 57.

(10) Imoto, M.; Yoshimura, H.; Kusumoto, S.; Shiba, T. *Proc. Jpn. Acad.* **1984**, *60*, 285.

(11) Imoto, M.; Yoshimura, H.; Yamamoto, M.; Shimamoto, T.; Kusumoto, S.; Shiba, T. *Tetrahedron Lett.* **1984**, *25*, 2667.

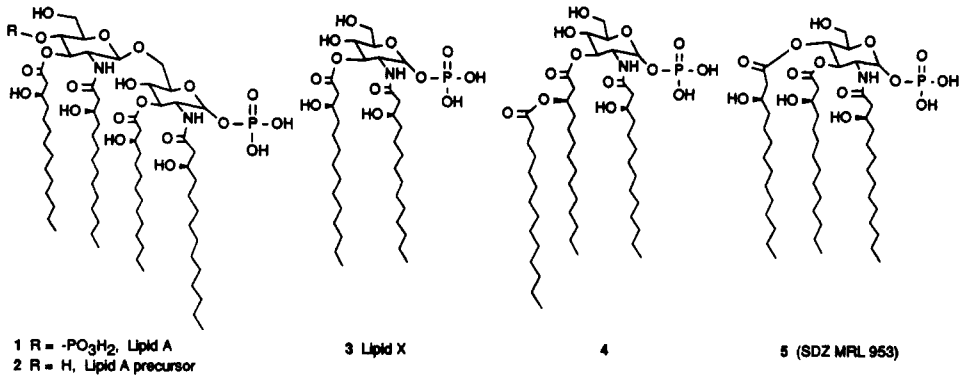
(12) Nakamoto, S.; Takahashi, T.; Ikeda, K.; Achiwa, K. *Chem. Pharm. Bull.* **1987**, *35*, 4517.

(13) Matsuura, M.; Kojima, M.; Homma, J. Y.; Kubota, Y.; Yamamoto, A.; Kiso, M.; Hasegawa, A. *FEBS Lett.* **1984**, *167*, 226.

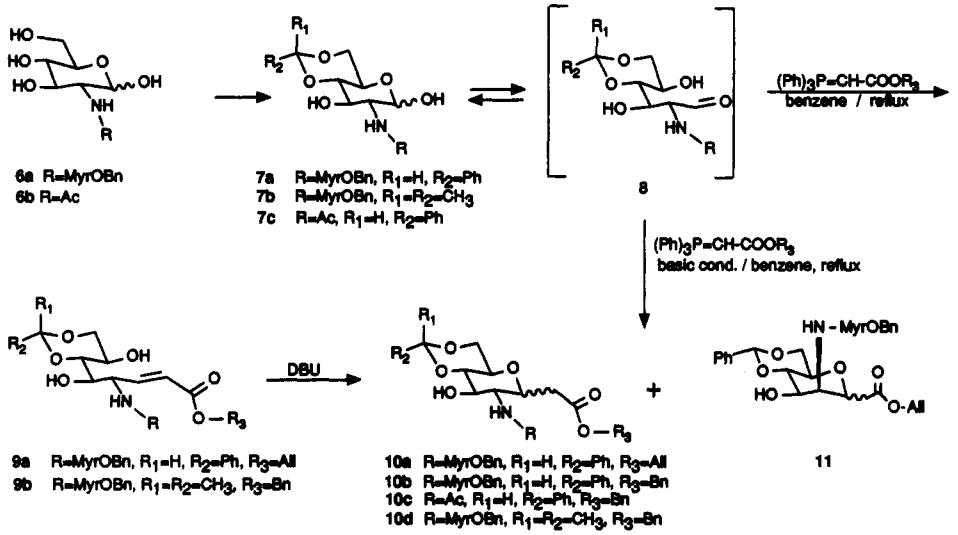
(14) Kumazawa, Y.; Matsuura, M.; Homma, J. Y.; Nakatsuru, Y.; Kiso, M.; Hasegawa, A. *Eur. J. Immunol.* **1985**, *15*, 199.

0022-2623/91/1834-2759$02.50/0

Chart I



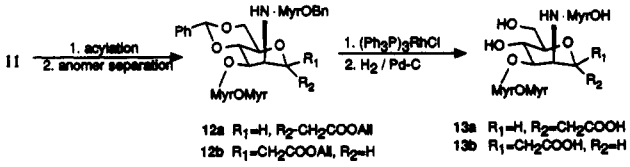
Scheme I. C-Glycosidation by Wittig Reaction



isolated from chemically manipulated or degraded LPSD preparations are prone to contamination by undetectable, but still bioactive, traces of LPS fragments, and standardization is thus extremely difficult. Consequently, our efforts have been directed toward the synthesis of chemically defined analogues of lipid A and its partial structures.

Until recently, the predominant paradigm was that lipid X (3), a 2,3-bis(3-hydroxymyristoylated) glucosamine 1-phosphate easily synthesized from glucosamine,[22] was the smallest substructure of lipid A still endowed with some of the immunostimulatory properties of LPS,[23-25] while

Scheme II. Preparation of the "Manno" Products

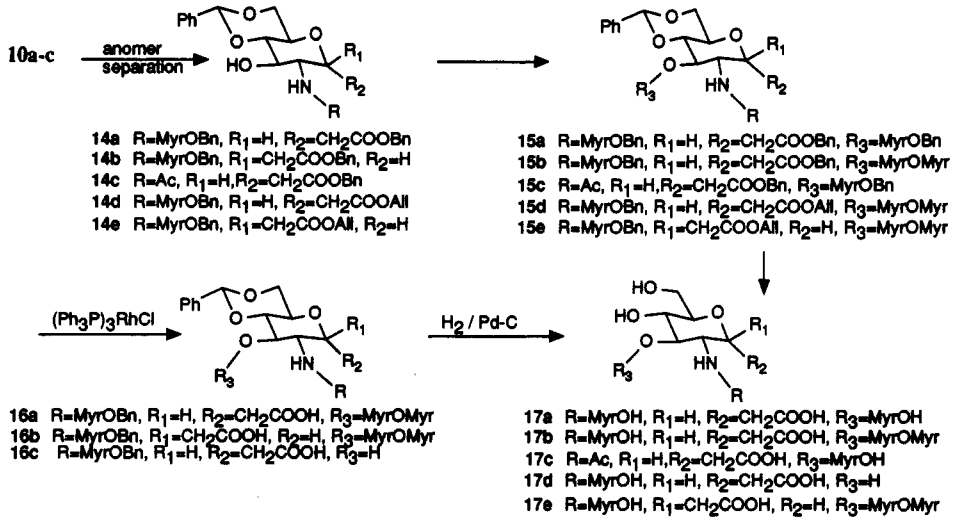


lacking any endotoxicity. However, we have recently demonstrated that the reported immunostimulatory activities of synthetic lipid X in fact resulted from contamination by small amounts of acylated β-(1→6)-glucosamine disaccharides.[26] The latter were formed during the last synthetic step, the catalytic hydrogenolysis of the benzyl protective groups of the 1-phosphate, presumably through activation of carbon 1 and condensation with a free hydroxyl in a Koenigs–Knorr type reaction.

As we have shown, highly purified synthetic lipid X is devoid of immunostimulatory activity[26] but is still able to antagonize certain effects of LPS in vitro and protects mice against a lethal dose of LPS if administered simultaneously.[27] After a thorough evaluation of structure–activity relationships of highly purified analogues of lipid X, it was found that the presence of three long-chain fatty acids per molecule is a prerequisite for immunostimulatory activity.[28,29] Compounds 4 and 5 are characteristic prototypes.

(15) Matsuura, M.; Yamamoto, A.; Kojima, Y.; Homma, J. Y.; Kiso, M.; Hasegawa, A. *J. Biochem. (Tokyo)* **1985**, *98*, 1229.
(16) Kumazawa, Y.; Matsuura, M.; Maruyama, T.; Kiso, M.; Hasegawa, A. *Eur. J. Immunol.* **1986**, *16*, 1099.
(17) Matsuura, M.; Kojima, Y.; Homma, J. Y.; Kumazawa, Y.; Yamamoto, A.; Kiso, M.; Hasegawa, A. *J. Biochem. (Tokyo)* **1986**, *99*, 1377.
(18) Raetz, C. R. H.; Purcell, A.; Takayama, K. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 4624.
(19) Nishijima, M.; Amano, F.; Akamatsu, A.; Akagawa, K.; Tokunaga, T.; Raetz, C. R. H. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 282.
(20) Shimizu, T.; Akiyama, S.; Masuzawa, T.; Yanighara, Y.; Nakamoto, S.; Takahashi, T.; Ikeda, K.; Achiwa, K. *Chem. Pharm. Bull. (Tokyo)* **1985**, *33*, 4621.
(21) Lasfargues, A.; Charon, D.; Tarigalo, F.; Ledu, A. L.; Szabo, L.; Chaby, R. *Cell. Immunol.* **1986**, *98*, 8.
(22) Macher, I. *Carbohydr. Res.* **1987**, *162*, 79.
(23) Nishijima, M.; Amano, F.; Akamatsu, Y.; Akegawa, K.; Tokunaga, T.; Raetz, C. R. H. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 282.
(24) Amano, F.; Nishijima, M.; Akamatsu, Y. *J. Immunol.* **1986**, *136*, 4122.
(25) Sayers, T. J.; Macher, I.; Chung, J.; Kugler, E. *J. Immunol.* **1987**, *138*, 2935.
(26) Aschauer, H.; Grob, A.; Hildebrandt, J.; Schütze, E.; Stütz, P. *J. Biol. Chem.* **1990**, *265*, 9159.
(27) Lam, C.; Hildebrandt, J.; Schütze, E.; Rosenwirth, B.; Proctor, R. A.; Haselberger, A.; Stütz, P., submitted to *J. Biol. Chem.*

Scheme III. Preparation of the Monosaccharides



Scheme IV. Alternative Route to the Monosaccharides

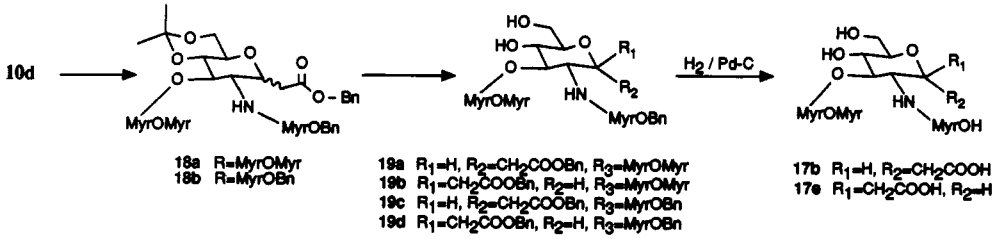
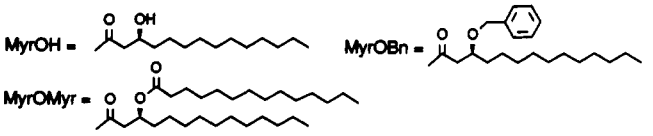


Chart II. Abbreviations Used in the Reaction Schemes

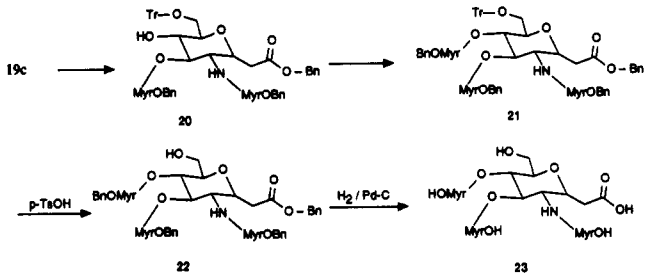


Compound 5 (SDZ MRL 953) shows a markedly increased therapeutic window in mice and has therefore been selected for clinical studies.[28-30]

An important aim of our work was to design analogues with improved chemical stability and to avoid the formation of unwanted disaccharidic artifacts during synthesis. By replacing the chemically unstable 1-*O*-phosphate by a "bioisosteric" 1-CH_2COOH group we expected to retain immunomodulatory properties while eliminating the formation of disaccharides by Koenigs–Knorr-like reactions. In addition this opened the route to further derivatization of position 1.

A key step of lipid A biosynthesis is the coupling of lipid X to UDP–lipid X by means of lipid A synthase produced by Gram-negative bacteria leading to the 4′-dephosphorylated lipid A precursor 2. This enzyme can be isolated from *Escherichia coli* and can be used for the preparation of a variety of artificial lipid A precursor analogues on preparative scale.[31-37] The low substrate specificity allows broad structural variation of both the reducing and nonreducing sugar moieties.[35b] In the following we report the synthesis of *C*-glycosidic mono- and disaccharide derivatives of lipid A and the evaluation of their immunostimulating and endotoxic properties.

Scheme V

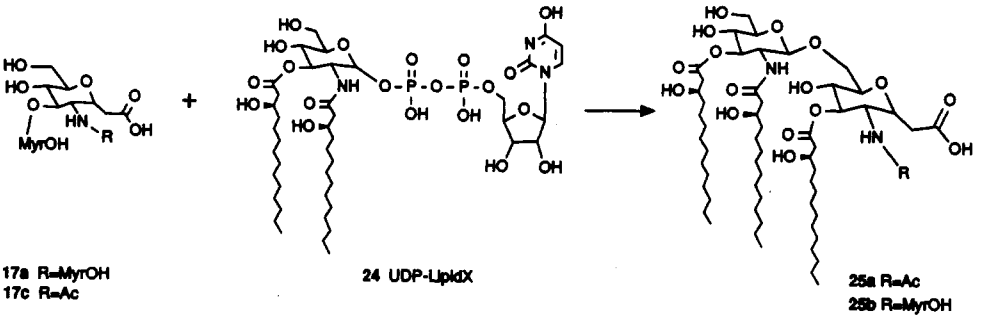


Results

Chemistry. Preparation of the Monosaccharides. The *C*-glycosidic monosaccharides were prepared as shown in Schemes I–VI (also see Chart II). Wittig reaction[38-42]

(28) Macher, I. European Pat. Appl. EP 309411 A2, 890329, 1989.
(29) Lam, C.; Schütze, E.; Hildebrandt, J.; Aschauer, H.; Liehl, E.; Macher, I.; Stütz, P. *Infect. Immun.*, in press.
(30) Lam, C.; Schütze, E.; Liehl, E.; Stütz, P. *Infect. Immun.*, in press.
(31) Bulawa, C. E.; Raetz, C. R. H. *J. Biol. Chem.* **1984**, *259*, 4846.
(32) Anderson, M. S.; Raetz, C. R. H. *J. Biol. Chem.* **1987**, *262*, 5159.
(33) Brozek, K. A.; Bulawa, C. E.; Raetz, C. R. H. *J. Biol. Chem.* **1987**, *262*, 5170.
(34) Anderson, M. S.; Bulawa, C. E.; Raetz, C. R. H. *J. Biol. Chem.* **1987**, *260*, 15536.
(35) Macher, I.; Unger, F. M.; Raetz, C. R. H. Germany Offen. DE 3621122 A1, 870122, 1987.
(36) Loibner, H.; Scholz, D.; Vyplel, H., unpublished results.
(37) Stütz, P.; Aschauer, H.; Hildebrandt, J.; Lam, C.; Loibner, H.; Macher, I.; Schütze, E.; Vyplel, H. In *Cellular and Molecular Aspects of Endotoxin Reactions*; Nowotny, A., Spitzer, J. J., Ziegler, E. J., Eds.; Elsevier: Amsterdam, 1990.
(38) Ray, B. L.; Painter, G.; Raetz, C. R. H. *J. Biol. Chem.* **1984**, *259*, 4852.
(39) Lerch, U.; Burdon, M. G.; Moffatt, J. G. *J. Org. Chem.* **1971**, *36*, 1507.
(40) Nicotra, F.; Russo, G.; Ronchetti, F.; Toma, T. *Carbohydr. Res.* **1983**, *124*, C5.
(41) Giannis, A.; Münster, P.; Sandhoff, K.; Steglich, W. *Tetrahedron* **1988**, *44*, 7177.

Scheme VI. Synthesis of the Disaccharides



of suitably protected glucosamine derivatives **7a–c** led to the intermediate olefins **9a,b**, which upon addition of DBU rapidly cyclized, forming the corresponding monosaccharides **10a–d**. With a carefully prepared Wittig reagent,[43] **9** could be isolated as a single (trans) isomer or cyclized in situ with DBU without purification. If the Wittig reagent was not thoroughly washed neutral prior to use, in order to remove traces of the base used in the preparation of the ylide, the yields were much lower. In this case, a byproduct (**11**) with "manno" configuration was isolated, probably resulting from a base-catalyzed racemization at the aldehyde stage **8**. The anomeric ratio (α:β) varied from 4:1 to 3:2. Separation of the anomeric mixtures **10a–d** and **13a,b** depended on the ester used as protective group for the carboxylic acid and on the sugar protective group. When the benzyl ester of the 4,6-*O*-benzylidene-protected sugar was used, the anomers could not be separated chromatographically and repeated crystallization from methanol was required to obtain only small amounts of the pure α-anomer **14a** containing less than 5% of the β-anomer **14b**, which in turn could not be crystallized. However, using the allyl in place of the benzyl ester, the two anomers could be completely separated by column chromatography on silica gel.

An alternative route is shown in scheme VI. When using a benzyl ester and 4,6-*O*-isopropylidene as protective groups (**10d**), the chromatographic separation of the anomeric mixture was easily achieved after acylation (**18**) and acidic cleavage of the 4,6-*O*-isopropylidene group (**19**). This route gave similar overall yields. The synthesis of the MRL 953 analogue **23** is outlined in scheme V. Tritylation of **19c** proceeded smoothly in pyridine using 4-Å molecular sieve as water scavenger. Subsequent acylation followed by detritylation (*p*-toluenesulfonic acid) and hydrogenolysis gave **23**. All acylations were performed with dicyclohexyl carbodiimide and catalytic amounts of 4-(dimethylamino)pyridine. The allyl esters were cleaved with chlorotris(triphenylphosphine)rhodium, and the benzyl groups were removed by catalytic hydrogenolysis. In order to ensure better water solubility all compounds to be tested were converted to their Tris salts. For all final reaction steps, pyrogen-free water was used for reaction and workup.

Preparation of the Disaccharides. Raetz[31–34] produced a strain of *E. coli* (MC 1061/p5r8, Δ 2515) which overproduces lipid A synthase. Extracts of this enzyme preparation can be used without extensive purification to couple UDP-lipid X (or even its analogues) with monosaccharides. This has provided a tool for the preparation of a variety of new, chemically homogeneous derivatives of lipid A precursors. In the case of slow-reacting substrates UDP-lipid X is cleaved by a hydrolase closely related to lipid A synthase affording lipid X. Thus, most of the UPD-derivative will be consumed and only minute amounts of the desired disaccharide are formed. In these cases another *E. coli* strain (JB 1104, Δ2514), which is devoid of this hydrolase activity, though not being an overproducer of lipid A synthase, was used with success.

Coupling of the *C*-glycosyl monosaccharides with UDP-lipid X was performed in normal laboratory glassware. After dissolving of the starting compounds in a buffer solution, a crude enzyme preparation was added and the mixture was incubated at 30 °C. After complete consumption of the UDP-lipid X (thin-layer chromatography) the product was isolated by a series of chromatographic steps.

Only compounds **17a** and **17c** gave the desired products **25a** (50% yield) and **25b** (58% yield), respectively, while all other compounds did not react. This is in accordance with our previous observations on the substrate specificity of lipid A synthase.[35–37] In order to achieve an acceptable yielcd, position 3 must be O-acylated, but with only one fatty acid, and the 4-hydroxy group must be free. Under identical reaction conditions (concerning enzyme batch, concentrations, temperature, salt and buffer concentration, etc.) the phosphorylated natural product **2** was formed 3 times faster than **25b**.

UDP-lipid X (**24**) was prepared according to the procedure described by Raetz.[34] Due to its instability it was used immediately without further purification. For biological testing the lysine salts of the disaccharides were prepared.

Biology. Identifying analogues of lipid A or LPS with an improved safety margin requires that beneficial and detrimental activities of these standards and of the experimental compounds are compared in suitable models. As a representative efficacy model for immunostimulatory activity we used the restoration of host resistance against a *Pseudomonas aeruginosa* septicemia in myelosuppressed mice[32,44,45] ("nonspecific resistance", NSR) after ip administration of lipid A analogues. First the survival rate was determined in a single dose experiment (12.5 mg/kg) followed by the evaluation of the ED_{50} for all compounds which induced a survival rate of more than 70%. Since endotoxicity cannot be measured easily in normal mice as they are rather insensitive to LPS, contrary to other animal species, e.g. sheep, the endotoxic potential (LD_{50}) of all immunostimulatory active compounds was determined by iv treatment of mice which were sensitized to LPS by a simultaneous administration of galactosamine.[46]

(42) Giannis, A.; Sandhoff, K. *Carbohydr. Res.* **1987**, *171*, 201.
(43) Isler, O.; Gutmann, H.; Montavon, M.; Rüegg, R.; Ryser, G.; Zeller, P. *Helv. Chim. Acta* **1957**, *40*, 1243.
(44) Parant, M. In *Beneficial Effects of Endotoxins*; Nowotny, A., Ed.; Plenum Press: New York, 1983; pp 179–196.
(45) Chase, J. J.; Kubey, W.; Dulek, M. H.; Holmes, C. J.; Salit, M. G.; Pearson, F. C.; Ribi, E. *Infect. Immun.* **1986**, *53*, 711.
(46) Galanos, C.; Freudenberg, M. A.; Reutter, W. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 5939.

Table I. Biological Activities of Lipid A/X Analogues

	NSR (ip)[a] survival rate	NSR (ip)[b] ED_{50}, mg/kg	ET shock (iv)[c] LD_{50}, mg/kg
3	0/10		
4	9/10	1.7 (1.2–2.4)	0.3 (0.1–0.6)
5	10/10	1.1 (0.8–1.4)	0.8 (0.6–1.0)
13a	4/10		
13b	2/10		
17a	4/10		
17b	9/10	1.8 (0.8–3.2)	0.2 (0.05–0.3)
17c	1/10		
17d	0/10		
17e	4/10		
23	10/10	1.1 (0.5–1.7)	1.0 (0.6–1.5)
2	8/10	3.4 (2.3–5.0)	3.5 (2.2–6.0)
25b	10/10	1.5 (1.1–2.1)	≈5.2
25a	7/10		
LPS	10/10	1.9×10^{-3} ($0.5–8.0 \times 10^{-3}$)	2×10^{-5} ($1–3 \times 10^{-5}$)

[a] NSR (nonspecific resistance against a *P. aeruginosa* (Δ12) infection): ip administration of a single dose (12.5 mg/kg) 1 day prior to infection, sample size 10 mice. [b] NSR, ip ED_{50} (confidence limits). [c] Endotoxin (ET) shock induction, LD_{50}, iv administration of substance + galactosamine (400 mg/kg).

The combined biological results for all compounds and for the standard LPS are given in Table I. The first column shows the survival rates of mice after single dose ip application of the respective compounds in the mouse infection model. The second column contains the ED_{50} values determined for all compounds with a survival rate greater than 70%. As is evident from the table, lipid X (**3**), containing only two fatty acids, has no immunostimulatory activity. On the other hand, both compounds **4**, where the 3-*O*-[(*R*)-3-hydroxymyristoyl] group is acylated by an additional myristic acid, and **5**, where a third *O*-[(*R*)-3-hydroxymyristoyl] group is attached to position 4, are fully protective in this model. All compounds with less than three myristic acids are inactive. Comparison of the ED_{50} values of column 2 shows that **5** is slightly more active than **4**. Replacement of the 1-phosphate by a CH_2COOH group led to compounds **17b** and **23**, which show virtually the same activities in the primary screen and in the ED_{50} evaluation as their phosphorylated counterparts. The same relationship holds true if one compares the endotoxin (ET) shock induction data of column 3: again LD_{50} values of the two pairs (**4**/**17b** and **5**/**23**) are nearly identical, although the *C*-glycosidic analogue **23** has a slightly better LD_{50} than the corresponding compound **5**, thus resulting in a slightly better overall therapeutic ratio.

On the other hand, stereochemistry seems to play an important role for activity: compound **17e**, the β-anomer of **17b**, and the diastereoisomeric compounds **13a,b** with manno configuration are only marginally active. These findings suggest that this region is important for recognition or binding, although there is no corresponding β-*O*-phosphate analogue available for comparison as such compounds are chemically not stable and the manno-type 1-phosphate analogues have not been synthesized.

A similar result can be seen for the disaccharides (**2** and **25a,b**; see lower part of the table): For instance, in the infection model the *C*-glycosidic analogue **25b** is as effective as its natural counterpart **2** and exhibits a rather low lethal toxicity in the GalN-sensitized mouse model. For comparison, LPS is 10^3 times more potent in the infection model but about 10^4 times more toxic.

(47) Finney, D. J. *Probit Analysis*, 3rd ed.; Cambridge University Press: Cambridge, MA, 1971.

Conclusions

With regard to biological activity, the anomeric phosphate group, both in the mono- and disaccharide series, can be replaced by a *C*-glycosidic acetic acid function without loss of immunostimulatory activity. At least three fatty acid residues per molecule appear to be necessary, as it was previously observed for 1-phosphorylated compounds.[26,27,29] Equally important is the stereochemistry at positions 1 and/or 2 as could be shown for the β-analogue **17a** and the diastereoisomeric compounds with 'manno' configuration **13a** and **13b**, which are all inactive. With regard to the endotoxic potential, the *C*-glycosidic analogues do not appear to offer significant advantages over the *O*-phosphates. The observed differences for the two pairs of prototypes are too small for drawing firm conclusions. However, both groups have a significantly higher therapeutic index in mice when compared to LPS from *S. abortus equi* used as laboratory standard.

Due to the low substrate specificity of lipid A synthase from *E. coli* strains, *C*-glycosidic disaccharide analogues of the lipid A precursor **2** can be prepared by enzymatic coupling of *C*-glycosidic "bioisosteric" analogues of lipid X with UDP-lipid X. As a prerequisite, the *C*-glycosidic mimic of the reducing sugar part must be acylated at positions 2 and 3.

Experimental Section

Chemistry. General Procedures. ^1H NMR spectra were recorded with a Bruker WM-250 Fourier transform, superconducting spectrometer at 250 MHz, with 32 scans, sweep width of 2.5 kHz, and 16000 data points. Chemical shifts are reported in ppm relative to internal Me_4Si. All *J* values are in hertz. The abbreviations indicating the multiplicity of the signal are s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet, b, broad. A prefix of d indicates "doublet of", e.g., dd = doublet of doublets. Optical rotations were determined with a Perkin-Elmer 141 polarimeter at 20 °C. Elemental analyses were determined by Dr. J. Zak (Mikroanalytisches Laboratorium am Institut für Physikalische Chemie, Universität Wien) and were within 0.4% of the calculated values unless stated otherwise. All mass spectra are fast atom bombardment (FAB) spectra. They were recorded on a VG 70-SE instrument (VG Analytical) operating at 8 kV accelerating voltage. The samples were applied in a thioglycerol/HCl matrix. Analytical thin-layer chromatography was performed on silica gel 60 F_{254} glass plates (Merck) with visualization by UV, I_2, or vanillin–sulfuric acid. Preparative column chromatography was performed on Merck-Lichroprep columns (silica gel, 40–63 μm) under pressure (~0.2 mPa). Reversed-phase chromatography was done on Merck-Lichroprep RP-18 (40–63 μm) material. Solvents were AR grade and were used without purification except pyridine used for chromatography, which was distilled over KOH. Pyrogen-free water was used for all final steps. Unless otherwise noted all reagents were obtained from commercial suppliers and were used without purification. All evaporations were carried out in vacuo with a rotary evaporator. No attempts were made to maximize the yields of the synthetic reactions.

Abbreviations used: DTE = dithioerythritol, EDTA = ethylenediaminotetraacetic acid, Tris = tris(hydroxymethyl)-aminomethane.

2-[(*R*)-3-(Benzyloxy)tetradecanamido]-2-deoxy-4,6-*O*-isopropylidene-D-glucopyranose (7b). A mixture of 2-[(*R*)-3-(benzyloxy)tetradecanamido]-2-deoxy-D-glucose (**6a**)[22] (35.8 g, 72.2 mmol), isopropyl methyl ether (14 mL, 144.4 mmol), 4-Å molecular sieve (64 g) and *p*-toluenesulfonic acid (200 mg) in dry dimethylformamide (640 mL) was stirred at room temperature for 1 h, then the mixture was filtered and evaporated. Chromatography with toluene–ethyl acetate (gradient from 3:2 to 1:1) afforded 32 g (60%) of **7b**: TLC R_f = 0.7 (chloroform–methanol 7:1); NMR ($CDCl_3$) (α:β ratio = 5:1) δ 7.35 (m, 5 H, H_{Ph}), 6.95 and 6.68 (2 × d, 1 H, NH (5:1), $J_{NH,2(\alpha)}$ = 6, $J_{NH,2(\beta)}$ = 7), 5.0 (t, 0.83 H, $H_{1(\alpha)}$, *J* = 4), 4.62 (4.63) and 4.46 (4.47) (2 × AB (5:1), 2 H, $PhCH_2O$, J_{AB} = 11), 4.08 (ddd, 1 H, H_2, *J* = 6.5, 9.5), 1.52 (1.53) (2 × s, CH_3), 1.42 (1.43) (2 × s, CH_3), 0.88 (t, 3 H, CH_3);

$[\alpha]^{20}_D$ +2.7° (c 10, CH_2Cl_2); MS m/e 536 $[MH]^+$, 518 $[M - OH]^+$, 496 $[M - (CH_3)_2C]^+$, 446 [M – benzyl], 181 $[M^+$ – side chains]. Anal. $C_{30}H_{49}NO_7$ (535.73) C, H, N.

Allyl 6,8-O-Benzylidene-4-[(*R*)-3-(benzyloxy)tetradecanamido]-2,3,4-trideoxy-D-*glyco*-oct-2-(*Z*)-enoate (9a). Glucosamine **7a** (1.14 g, 1.95 mmol) and [(allyloxy)carbonyl]methyltriphenylphosphonium ylide[39] (800 mg, 2.22 mmol) in dry acetonitrile (10 mL) were heated at reflux for 24 h. The solvent was then evaporated. Chromatography of the residue with toluene–ethyl acetate (2:1) afforded 360 mg (28%) of a colorless oil: TLC R_f = 0.72 (toluene–ethyl acetate 1:1); NMR ($CDCl_3$) δ 7.3 (m, 10 H, H_{Ph}), 7.0 (dd, 1 H, H_3, $J_{3,4}$ = 5.5, $J_{2,3}$ = 16), 6.93 (d, 1 H, NH, $J_{NH,4}$ = 8.5), 6.02 (dd, 1 H, H_2, $J_{2,4}$ = 2.5, $J_{2,3}$ = 16), 5.9 (m, 1 H, $CH{=}C_{Allyl}$), 5.37 (s, 1 H, PhCH), 5.25 (m, 2 H, $C{=}CH_2$), 5.16 (bm, 1 H, H_4), 4.64 (dt, 2 H, $OCH_2C{=}$, J = 5, J = 1.5), 4.50, 4.51 (AB, 2 H, OCH_2Ph, J_{AB} = 12), 4.25 (dd, 1 H, H_{8e}, $J_{8e,7}$ = 5, $J_{8e,8a}$ = 10), 4.0 (m, 1 H, H_5), 3.83 (m, 2 H, H_7, CHOBn), 3.5 (m, 2 H, H_4, H_{6a}), 0.90 (t, 3 H, CH_3); $[\alpha]^{20}_D$ –8.3° (c 10, $CHCl_3$); MS m/e 666 $[MH]^+$, 560 $[M - PhCHO]^+$. Anal. $C_{39}H_{55}NO_8$ (665.88) C, H, N.

Benzyl 4-[(*R*)-3-(Benzyloxy)tetradecanamido]-6,8-O-isopropylidene-2,3,4-trideoxy-D-*glyco*-oct-2-(*Z*)-enoate (9b). Glucosamine **7b** (82 mg, 0.15 mmol) and [(benzyloxy)carbonyl]methyltriphenylphosphonium ylide[39] (120 mg, 0.3 mmol) were refluxed overnight in dry benzene (25 mL). The solvent was evaporated and the residue chromatographed with toluene–ethyl acetate (3:1), affording 15 mg (15%) of a colorless oil: TLC R_f = 0.5 (hexane–ethyl acetate 1:2); NMR ($CDCl_3$) δ 7.3 (m, 10 H, H_{Ph}), 6.95 (dd, 1 H, H_3, $J_{3,4}$ = 5.5, $J_{2,3}$ = 16), 6.92 (d, 1 H, NH, $J_{NH,4}$ = 8), 6.03 (dd, 1 H, H_2, $J_{2,4}$ = 2.5, $J_{2,3}$ = 16), 5.18 (s, 2 H, $COOCH_2Ph$), 5.01 (bm, 1 H, H_4), 4.58, 4.31 (AB, 2 H, $PhCH_2OC$, J_{AB} = 11.5), 3.9–3.5 (m, 7 H), 0.9 (t, 3 H, CH_3); $[\alpha]^{20}_D$ –7.2° (c 10, CH_2Cl_2); MS m/e 668 $[MH]^+$, 610 $[M$ – acetone$]^+$. Anal. $C_{39}H_{57}NO_8$ (667.89) C, H, N.

Allyl [4,6-O-benzylidene-2-[(*R*)-3-(benzyloxy)tetradecanamido]-2-deoxy-α,β-D-glucopyranosyl]acetate (10a) was first prepared by treating **7a** (15 g, 26.3 mmol) in benzene (1400 mL) at reflux temperature with unpurified [(allyloxy)carbonyl]methyltriphenylphosphonium ylide (19.93 g, 55.3 mmol). After 20 h the solvent was evaporated and the residue chromatographed with toluene–ethyl acetate (gradient 3:1 to 1:1) to yield two fractions: 5.85 g (33%) of **10a** and 7.68 g (44%) of a white solid which was characterized as the 2-epimer product **11** (see below).

10a: TLC R_f = 0.57 (toluene–ethyl acetate 1:1); α:β ratio = 4:1 (according to NMR); NMR ($CDCl_3$) δ NH at 6.62 and 6.43 (2 × d, 1 H, (4:1), $J_{NH,2}$ = 7(α) and 8.5(β)); $[\alpha]^{20}_D$ +24.1° (c 10, CH_2Cl_2).

The overall yield could be optimized by using the following procedure: **7a** (15.3 g, 28.9 mmol) and the rigorously purified Wittig reagent (15.5 g, 43.1 mmol) in benzene (1400 mL) were stirred for 24 h under reflux. The solvent was evaporated, the residue redissolved in dichloromethane (500 mL), and the solution treated with DBU (2 mL). After stirring for 1 h the mixture was washed with saturated $KHSO_4$ solution and evaporated. The residue was chromatographed (toluene–ethyl acetate 3:1) providing **14d** (7.64 g, 43%), **14e** (1.27 g, 7%), and an impure fraction (α:β 2:1, 1.7 g, 9.5%). This amounts to an overall yield of 59%.

α-Anomer **14d**: TLC R_f = 0.42 (hexane–ethyl acetate 1:1); NMR ($CDCl_3$) δ 7.4 (m, 10 H, H_{Ph}), 6.6 (d, 1 H, NH, $J_{NH,2}$ = 7), 5.9 (m, 1 H, $CH{=}C$), 5.52 (s, 1 H, PhCH), 5.3 (m, 2 H, $C{=}CH_2$), 4.76 (ddd, 1 H, H_1, $J_{1,2}$ = 6, $J_{1,1A}$ = 5, $J_{1,1B}$ = 12), 4.6 (m, 4 H, $PhCH_2O$, $OCH_2C{=}C$), 4.2 (m, 2 H, H_2, H_6), 3.84 (m, 1 H, H_3), 2.86 (d, 1 H, OH, J = 3), 0.88 (t, 3 H, CH_3); $[\alpha]^{20}_D$ +22.5° (c 10, $CHCl_3$); MS m/e 666 $[MH]^+$, 626 $[M$ – allyl$]^+$, 560 $[M - PhCH_2O]$, 334 [M – RCON]. Anal. $C_{39}H_{55}NO_8$ (665.88) C, H, N.

β-Anomer **14e**: TLC R_f = 0.55 (cyclohexane–ethyl acetate 1:1); NMR ($CDCl_3$) δ 7.4 (m, 10H, H_{Ph}), 6.38 (d, 1 H, NH, $J_{NH,2}$ = 8.5), 5.9 (m, 1 H, $CH{=}C$), 5.53 (s, 1 H, PhCH), 5.28 (m, 2 H, $C{=}CH_2$), 4.62, 4.46 (AB, 2 H, $PhCH_2O$, J_{AB} = 10.5), 4.58 (m, 2 H, $CH_2C{=}C$), 4.28 (ddd, 1 H, H_{6e}, $J_{6e,6a}$ = 10, $J_{6e,5}$ = 5), 0.88 (t, 3 H, CH_3); $[\alpha]^{20}_D$ –26.0° (c 10, $CHCl_3$); MS m/e 666 $[MH]^+$, 578 $[M - PhCH_2]^+$, 560 $[M - PhCO]^+$. Anal. $C_{39}H_{55}NO_8$ (665.88) C, H, N.

Benzyl [4,6-O-benzylidene-2-[(*R*)-3-(benzyloxy)tetradecanamido]-2-deoxy-α,β-D-glucopyranosyl]acetate (10b) was prepared as described for **10a** from glucosamine derivative **7a** and [(benzyloxy)carbonyl]methyltriphenylphosphonium ylide. After chromatography (toluene–ethyl acetate 1:1) a pale yellow oil was obtained in 33% yield which solidified on cooling: TLC R_f = 0.6 (toluene–ethyl acetate 1:1); NMR ($CDCl_3$) indicated an α:β ratio of 3:2 [according to NH at 6.57 and 6.38 (2 × d, 1H, (3:2), $J_{NH,2}$ = 7 and 8.5)].

Separation of anomers was achieved by repeated crystallization from methanol affording small amounts of **14a** (α-anomer, >95% pure) and **14b** (β-anomer, 2:1 α:β mixture, not further separated) as white solids.

α-Anomer **14a**: TLC R_f = 0.6 (chloroform–methanol 95:5); NMR ($CDCl_3$) δ 7.5–7.2 (m, 15 H, H_{Ph}), 6.56 (d, 1 H, NH, $J_{NH,2}$ = 7), 5.5 (s, 1 H, PhCH), 5.12 (s, 2 H, $PhCH_2OC$), 4.78 (ddd, 1 H, H_1, $J_{1,2}$ = $J_{1,1A}$ = 6, $J_{1,1B}$ = 6), 4.6, 4.42 (AB, 2 H, $PhCH_2O$, J_{AB} = 11.5), 4.2 (dt, 1 H, H_2, $J_{2,NH}$ = 7, $J_{2,3}$ = 10, $J_{2,1}$ = 6), 4.1 (m, 1 H, H_{6e}), 3.8 (m, 1 H, H_3), 3.58 (m, 3 H, H_4, H_5, H_{6a}), 0.88 (t, 3 H, CH_3); $[\alpha]^{20}_D$ +21.3° (c 10, $CHCl_3$); MS m/e 716 $[MH]^+$, 672, 626 $[M - PhCH]^+$, 610 $[M - PhCH_2O]^+$. Anal. $C_{43}H_{57}NO_8$ (715.9) C, H, N.

β-Anomer **14b**: TLC R_f = 0.59 (toluene–ethyl acetate 1:1); NMR ($CDCl_3$) δ 7.4 (m, 15 H, H_{Ph}), 6.58 and 6.38 (2 × d, (α:β 1:2), NH, $J_{NH,2(\alpha\text{-anom})}$ = 6.5, $J_{NH,2(\beta\text{-anom})}$ = 8), 5.5 (s, 1 H, PhCH), 5.12 (2 × s, (2:1), 2 H, $PhCH_2O$), 4.88 (m, 0.3 H, $H_{1\beta}$), 4.6–4.4 (m, 2 H, $PhCH_2O$), 0.87 (t, 3 H, CH_3); $[\alpha]^{20}_D$ –6.7° (c 10, $CHCl_3$). Anal. $C_{43}H_{57}NO_8$ (715.9) C, H, N.

Benzyl [2-acetamido-4,6-O-benzylidene-2-deoxy-α,β-D-glucopyranosyl]acetate (10c) was prepared as anomer mixture (α:β ratio 2:1) from the protected *N*-acetylglucosamine **7c** in 37% yield by using the procedure described for **10a**. Repeated crystallization from methanol afforded benzyl [2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranosyl]acetate (**14c**): TLC R_f = 0.3 (dichloromethane–methanol 9:1); NMR δ ($CDCl_3$–CD_3OD) 7.4 (m, 10 H, H_{Ph}), 5.53 (s, 1 H, PhCH), 5.17, 5.15 (AB, 2 H, $PhCH_2O$, J_{AB} = 12), 4.79 (ddd, 1 H, H_1, $J_{1,2}$ = 6, $J_{1,1A}$ = 11, $J_{1,1B}$ = 5), 4.17 (dd, 1 H, H_2, $J_{2,1}$ = 6, $J_{2,3}$ = 10.5), 4.03 (m, 1 H, H_{6e}), 3.8 (dd, 1 H, H_3, $J_{3,2}$ = 10.5, $J_{3,4}$ = 9), 1.97 (s, 3 H, CH_3); $[\alpha]^{20}_D$ +44.8° (c 10, $CHCl_3$–CH_3OH 9:1); MS m/e 442 $[MH]^+$, 400 $[M - CH_3CO]^+$, 354 $[M - PhCH]^+$, 336 $[M - PhCHO]$. Anal. $C_{24}H_{27}NO_7$ (441.49) C, H, N.

Benzyl [2-[(*R*)-3-(Benzyloxy)tetradecanamido]-2-deoxy-4,6-O-isopropylidene-α,β-D-glucopyranosyl]acetate (10d). Treatment of **7b** with [(benzyloxy)carbonyl]methyltriphenylphosphonium ylide, using the procedure described for **10a**, afforded after chromatography (toluene–ethyl acetate 2:1) a colorless wax in 46% yield as a mixture of anomers (α:β ratio = 3:1): TLC R_f = 0.57 (toluene–ethyl acetate 1:1); NMR ($CDCl_3$) δ 7.3 (m, 10 H, H_{Ph}), 6.58 and 6.38 (2 × d (3:1), 1 H, NH, $J_{NH,2(\alpha\text{-anom})}$ = 7, $J_{NH,2(\beta\text{-anom})}$ = 8), 5.11 , 5.10 (2 × s, 2 H (1:3), $PhCH_2O$), 4.72 (ddd, 0.75 H, $H_{1\beta}$, $J_{1\beta,2}$ = 6, $J_{1\beta,1A}$ = 11.25, $J_{1,1B}$ = 5), 4.61 (4.60) and 4.44 (4.43) (2 × AB (1:3), 2 H, $PhCH_2O$, J_{AB} = 12), 4.16 (m, 1 H, H_2), 1.28 (1.29) (2 × s (1:3), 3 H, CH_3), 1.21 (1.20) (2 × s (1:3), 3 H, CH_3), 0.88 (t, 3 H, CH_3); $[\alpha]^{20}_D$ +8.4° (c 10, $CHCl_3$); MS m/e 668 $[MH]^+$, 610 [M – acetone], 520 [M – $(CH_3)_2CO$–benzyl]. Anal. $C_{39}H_{57}NO_8$ (667.89) C, H, N.

Allyl [4,6-O-benzylidene-2-[(*R*)-3-(benzyloxy)tetradecanamido]-2-deoxy-α,β-D-mannopyranosyl]acetate (11) was obtained as a byproduct during the preparation of **10b**: TLC R_f = 0.4 (toluene–ethyl acetate 1:1); NMR ($CDCl_3$) (1:1 mixture of anomers) δ 7.45 (m, 10 H, H_{Ph}), 7.19 and 7.03 (2 × d, 1 H (1:1), NH, $J_{NH,2(\alpha\text{-anom})}$ = 7.5, $J_{NH,2(\beta\text{-anom})}$ = 9.5), 6.0–5.8 (m, 1 H, $CH{=}C_{(allyl)}$), 5.3 (m, 2 H, $C{=}CH_2$), 5.09, 5.02 (2 × s (1:1), 1 H, PhCH), 4.6 (m, 5 H, $PhCH_2O$, $OCH_2C{=}C$, H_2), 4.4 (m, 0.5 H, $H_{1\beta}$), 3.88 (m, 1 H, CHO-benzyl), 0.9 (t, 3 H, CH_3); $[\alpha]^{20}_D$ –7.3° (c 10, CH_2Cl_2); MS m/e 666 $[MH]^+$, 560 [M – PhCO], 279. Anal. $C_{39}H_{55}NO_8$ (665.88) C, H, N.

Allyl [4,6-O-Benzylidene-2-[(*R*)-3-(benzyloxy)tetradecanamido]-2-deoxy-3-O-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-mannopyranosyl]acetate (12). A solution of **11** (2.66 g, 4 mmol) in dichloromethane (100 mL) at room temperature was treated with (*R*)-3-(tetradecanoyloxy)tetradecanoic acid[48] (2.0 g, 4.4 mmol), dicyclohexylcarbodiimide (910 mg, 4.4 mmol), and 4-(dimethylamino)pyridine (10 mg). After 20 h the mixture was filtered and the solvent evaporated. Chromatography (toluene–

(48) Kiso, M.; Tanaka, S.; Fujita, M.; Fujishima, Y.; Ogawa, Y.; Ishida, H.; Hasegawa, A. *Carbohydr. Res.* **1987**, *162*, 127.

ethyl acetate 20:1) gave 700 mg (16%) of the α-anomer (**12a**) and 460 mg (10%) of the β-anomer (**12b**).

12a: TLC R_f = 0.48 (toluene–ethyl acetate 4:1), NMR ($CDCl_3$) δ 7.35 (m, 10 H, H_{Ph}), 6.84 (d, 1 H, NH, $J_{NH,2}$ = 10), 5.9 (m, 1 H, CH=C), 5.3 (m, 2 H, C=CH_2), 5.15 (m, 2 H, H_3, CHOCO), 4.88 (s, 1 H, PhCH), 4.72 (s, 2 H, OCH_2Ph), 4.7 (m, 1 H, H_2), 4.62 (m, 2 H, CH_2C=C), 4.22 (m, 1 H, H_1), 4.08 (dd, 1 H, H_{6e}, $J_{6e,5}$ = 5, $J_{6e,6a}$ = 10), 3.86 (m, 1 H, CHOBn), 3.43 (dd, 1 H, H_5, $J_{5,4}$ = 10), 3.35 (t, 1 H, H_{6a}, J = 10), 3.17 (t, 1 H, H_4, J = 10), 0.9 (t, 9 H, 3 × CH_3); $[\alpha]^{20}_D$ −26.7° (*c* 10, $CHCl_3$); MS *m/e* 1102 $[MH]^+$, 996 $[M - PhCO]^+$, 666 [M − MyrOMyr]. Anal. $C_{67}H_{107}NO_{11}$ (1102.6) C, H, N.

12b: TLC R_f = 0.42 (toluene–ethyl acetate 1:1), NMR ($CDCl_3$) δ 7.35 (m, 10 H, H_{Ph}), 7.1 (d, 1 H, NH, $J_{NH,2}$ = 9.5), 5.9 (m, 1 H, CH=C), 5.32 (m, 2 H, C=CH_2), 5.24 (m, 2 H, CHOCO, H_3), 4.93 (s, 1 H, PhCH), 4.7 (s, 2 H, $PhCH_2O$), 4.6 (m, 2 H, CH_2C=C), 4.58 (ddd, 1 H, H_2, $J_{2,NH}$ = 9.5, $J_{2,3}$ = 3.5, $J_{2,1}$ = 2.5), 4.4 (dd, 1 H, H_1, $J_{1,1A}$ = 10, $J_{1,1B}$ = 5), 4.02 (dd, 1 H, H_{6e}, $J_{6e,6a}$ = 10, $J_{6e,5}$ = 5), 3.86 (m, 1 H, CHOBn), 3.72 (ddd, 1 H, H_5, $J_{5,4}$ = 10, $J_{5,6a}$ = 5, $J_{5,6e}$ = 10), 3.46 (dd, 1 H, H_{6a}, $J_{6a,6e}$ = 10, $J_{6a,5}$ = 10), 3.23 (t, 1 H, H_4, J = 10), 0.9 (t, 9 H, 3 × CH_3); $[\alpha]^{20}_D$ −15.0° (*c* 10, $CHCl_3$). Anal. $C_{67}H_{107}NO_{11}$ (1102.6) C, H, N.

[2-[(*R*)-3-Hydroxytetradecanamido]-2-deoxy-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-mannopyranosyl]acetic Acid (13a). A solution of **12a** (592 mg, 0.54 mmol) in refluxing ethanol (50 mL) was treated with chlorotris(triphenylphosphine)rhodium (99 mg, 0.1 mmol). After 5 days the reaction was complete (TLC) and the solvent was evaporated. The crude product was taken up in tetrahydrofuran (100 mL), treated with palladium (10% on charcoal), and hydrogenolyzed at room temperature for 24 h. Chromatography (chloroform–methanol–acetic acid 14:1:0.1) afforded 41 mg (9%) of a white solid: TLC R_f = 0.6 (chloroform–methanol–water–acetic acid 80:8:1:1); NMR ($CDCl_3$–CD_3OD 1:1) δ 5.23 (dt, 1 H, CHOCO), 4.9 (dd, 1 H, H_3, $J_{3,2}$ = 4.0, $J_{3,4}$ = 10), 4.54 (d, b, 1 H, H_2), 4.14 (m, 1 H, H_1), 3.96 (m, 1 H, H_5), 3.84 (d, 2 H, H_6), 3.76 (t, 1 H, H_4, $J_{4,5}$ = 10), 0.9 (t, 9 H, 3 × CH_3); MS *m/e* 884 $[MH]^+$, 448 [M − MyrOMyr]. Anal. $C_{50}H_{93}NO_{11}$ (884.30) C, H, N.

[2-[(*R*)-3-Hydroxytetradecanamido]-2-deoxy-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-mannopyranosyl]acetic acid (13b) was prepared in 23% yield following the procedure described for **13a**: TLC R_f = 0.27 (chloroform–methanol–water–acetic acid 80:8:1:1); NMR ($CDCl_3$–CD_3OD 1:1) δ 5.23 (dt, 1 H, CHOCO), 5.0 (dd, 1 H, H_3, $J_{3,2}$ = 4.5, $J_{3,4}$ = 8), 4.47 (t, 1 H, H_2, $J_{2,3}$ = 4.5, $J_{2,1}$ = 4.5), 4.31 (m, 1 H, H_1), 0.9 (t, 9 H, 3 × CH_3); MS *m/e* 884 $[MH]^+$, 448 [M − MyrOMyr]. Anal. $C_{50}H_{93}NO_{11}$ (884.30) C, H, N.

Benzyl [4,6-*O*-benzylidene-2-[(*R*)-3-(benzyloxy)tetradecanamido]-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-α-D-glucopyranosyl]acetate (15a) was prepared from **14a** in 78% yield by using the acylation procedure described for **12a** (chromatography with toluene–ethyl acetate 4:1): TLC R_f = 0.54 (toluene–ethyl acetate 4:1); NMR ($CDCl_3$) (contains ca. 5% β-anomer) δ 7.3 (m, 20 H, H_{Ph}), 6.38 (d, 1 H, NH, $J_{NH,2}$ = 7.5), 5.45 (s, 1 H, PhCH), 5.16 (m, 1 H, H_3), 5.1 (s, 2 H, $PhCH_2OCO$), 4.8 (ddd, 1 H, H_1, $J_{1,2}$ = 6.5, $J_{1,1A}$ = 5, $J_{1,1B}$ = 6.5), 4.6–4.4 (m, 5 H, 2 × $PhCH_2O$, H_2), 4.03 (m, 1 H, H_{6e}), 3.8–3.6 (m, 5 H, $PhCH_2O$, H_{6a}, H_5, H_4), 0.88 (t, 6 H, 2 × CH_3); $[\alpha]^{20}_D$ +20.8° (*c* 10, CH_2Cl_2); MS *m/e* 1032 $[MH]^+$, 944 $[M - PhCH]^+$, 926 [944 − H_2O], 716 $[M - MyrOBn]^+$, 698 [716 − H_2O]. Anal. $C_{64}H_{89}NO_{10}$ (1032.42) C, H, N.

Benzyl [4,6-*O*-benzylidene-2-[(*R*)-3-(benzyloxy)tetradecanamido]-2-deoxy-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranosyl]acetate (15b) was prepared in 40% yield following the procedure described above (chromatography with toluene–ethyl acetate 4:1): TLC R_f = 0.6 (toluene–ethyl acetate 4:1); NMR ($CDCl_3$) δ 7.3 (m, 15 H, H_{Ph}), 5.52 (d, 1 H, NH, $J_{NH,2}$ = 7), 5.48 (s, 1 H, PhCH), 5.2 (m, 4 H, H_3, CHOCO, $PhCH_2CO$), 4.85 (ddd, 1 H, H_1, $J_{1,2}$ = 6.5, $J_{1,1A}$ = $J_{1,1B}$ = 6), 4.57, 4.44 (AB q, 2 H, $PhCH_2O$, J_{AB} = 12), 4.37 (dt, 1 H, H_2, $J_{2,3}$ = 11.5), 4.02 (m, 1 H, H_{6e}), 3.82 (dt, 1 H, CHO, J = 11.5, 5), 0.88 (t, 9 H, 3 × CH_3); $[\alpha]^{20}_D$ +16.3° (*c* 10, CH_2Cl_2); MS *m/e* 1152 $[MH]^+$, 661, 536, 308. Anal. $C_{71}H_{109}NO_{11}$ (1152.66) C, H, N.

Benzyl [2-Acetamido-4,6-*O*-benzylidene-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-α-D-glucopyranosyl]acetate (15c). Acylation was performed as described for **12a**: yield 80% after chromatography (toluene–ethyl acetate 1:1); TLC R_f = 0.57 (toluene–ethyl acetate 1:1); NMR ($CDCl_3$) δ 7.3 (m, 15 H, H_{Ph}), 5.9 (d, 1 H, NH, $J_{NH,2}$ = 7), 5.49 (s, 1 H, PhCH), 5.2 (m, 1 H, H_3), 5.16 (d, 2 H, $PhCH_2OCO$, J = 1.2), 4.84 (dt, 1 H, H_1, $J_{1,2}$ = 6, $J_{1,1A}$ = 6, $J_{1,1B}$ = 10), 4.52, 4.43 (AB q, 2 H, $PhCH_2O$, J_{AB} = 11), 4.4 (ddd, 1 H, H_2, $J_{2,1}$ = 6, $J_{2,3}$ = 6, $J_{2,NH}$ = 7), 4.1 (b, 1 H, H_{6e}), 3.7 (m, 5 H, $PhCH_2O$, H_4, H_5, H_{6a}), 1.81 (s, 3 H, CH_3CON), 0.89 (t, 3 H, CH_3); $[\alpha]^{20}_D$ +31.7° (*c* 10, $CHCl_3$); MS *m/e* 758 $[MH]^+$, 720, 442 $[M - MyrOBn]^+$. Anal. $C_{45}H_{59}NO_9$ (757.97) C, H, N.

Allyl [4,6-*O*-Benzylidene-2-[(*R*)-3-(benzyloxy)tetradecanamido]-2-deoxy-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranosyl]acetate (15d). Acylation of **14d** as described above gave **15d** in 80% yield after chromatography with toluene–ethyl acetate 6:1: TLC R_f = 0.6 (toluene–ethyl acetate 4:1); NMR ($CDCl_3$) δ 7.35 (m, 10 H, H_{Ph}), 6.54 (d, 1 H, NH, $J_{NH,2}$ = 7), 5.85 (m, 1 H, CH=C), 5.51 (s, 1 H, PhCH), 5.25 (m, 2 H, C=CH_2), 5.15 (m, 2 H, CHOCO, H_3), 4.84 (ddd, 1 H, H_1, $J_{1,2}$ = $J_{1,1A}$ = 6, $J_{1,1B}$ = 12), 4.55 (m, 4 H, $PhCH_2O$, OCH_2C=C), 4.38 (dt, 1 H, H_2, $J_{2,3}$ = 11, $J_{2,1}$ = 6, $J_{2,NH}$ = 7), 4.2 (dt, b, 1 H, H_{6e}), 3.84 (dt, 1 H, CHOBn), 0.88 (t, 9 H, 3 × CH_3); $[\alpha]^{20}_D$ −18.1° (*c* 10, CH_2Cl_2); MS *m/e* 1102 $[MH]^+$, 994 $[M - PhCH_2OH]^+$, 536, 433, 308. Anal. $C_{67}H_{107}NO_{11}$ (1102.60) C, H, N.

Allyl [4,6-*O*-benzylidene-2-[(*R*)-3-(benzyloxy)tetradecanamido]-2-deoxy-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]acetate (15e) was prepared from **14e** in 53% yield after chromatography with toluene–ethyl acetate 9:1: TLC R_f = 0.63 (toluene–ethyl acetate 4:1); NMR ($CDCl_3$) δ 7.4 (m, 10 H, H_{Ph}), 6.16 (d, 1 H, NH, $J_{NH,2}$ = 9.5), 5.9 (m, 1 H, CH=C), 5.49 (s, 1 H, PhCH), 5.23 (m, 2 H, C=CH_2), 5.14 (m, 1 H, OCOCH), 5.05 (t, 1 H, H_3, J = 10), 4.6, 4.43 (AB, 2 H, $PhCH_2O$, J_{AB} = 11.5), 4.54 (dt, 2 H, CH_2C=C, J = 1.2 and 5.5), 4.28 (dd, 1 H, H_{6e}, $J_{6e,6a}$ = 10, $J_{6e,5}$ = 5), 4.08 (q, 1 H, H_2, $J_{2,NH}$ = $J_{2,3}$ = 9.5), 3.83 (m, 1 H, CHOBn), 3.7 (t, 1 H, H_{6a}, J = 9.5), 3.68 (dt, 1 H, H_1, $J_{1,2}$ = $J_{1,1A}$ = 6, $J_{1,1B}$ = 10), 3.63 (t, 1 H, H_4, J = 9.5), 3.44 (ddd, 1 H, H_5, J = 5, 10), 0.88 (t, 9 H, 3 × CH_3); $[\alpha]^{20}_D$ +13.5° (*c* 10, $CHCl_3$); MS *m/e* 1102 $[MH]^+$, 996 $[M - Ph - CH_2O]^+$, 876, 556, 209. Anal. $C_{67}H_{107}NO_{11}$ (1102.6) C, H, N.

[4,6-*O*-Benzylidene-2-[(*R*)-3-(benzyloxy)tetradecanamido]-2-deoxy-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranosyl]acetic acid (16a) was obtained from **15d** by using the procedure described for **13a**. Chromatography (chloroform–methanol 9:1) afforded **16a** as a white solid in 64% yield: TLC R_f = 0.6 (chloroform–methanol 7:1); NMR ($CDCl_3$–CD_3OD 1:1) δ 7.4 (m, 10 H, H_{Ph}), 5.55 (s, 1 H, PhCH), 5.29 (dd, 1 H, H_3, $J_{3,4}$ = 12, $J_{3,2}$ = 10), 5.17 (m, 1 H, HCOCO), 4.72 (dt, 1 H, H_1, $J_{1,2}$ = $J_{1,1A}$ = 6.5, $J_{1,1B}$ = 12), 4.54 (s, 2 H, $PhCH_2O$), 4.46 (dd, 1 H, H_2, $J_{2,1}$ = 6.5, $J_{2,3}$ = 10), 4.22 (dd, 1 H, H_{6e}, $J_{6e,6a}$ = 9, $J_{6e,5}$ = 4), 0.9 (t, 9 H, 3 × CH_3); $[\alpha]^{20}_D$ +16.5° (*c* 10, $CHCl_3$–CH_3OH 1:1); MS *m/e* 1062 $[MH]^+$, 974 $[M - PhCH_2]^+$, 757. Anal. Calcd for $C_{64}H_{103}NO_{11}$ (1062.53): C, 72.35; H, 9.77; N, 1.32. Found: C, 71.40; H, 8.89; N, 1.06.

[4,6-*O*-Benzylidene-2-[(*R*)-3-(benzyloxy)tetradecanamido]-2-deoxy-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]acetic Acid (16b). Cleavage of the allyl ester **15e** was carried out as described for **13a**, affording **16b** after chromatography (chloroform–methanol 9:1) in 64% yield: TLC R_f = 0.9 (chloroform–methanol 7:1); NMR ($CDCl_3$–CD_3OD 1:1) δ 7.4 (m, 5 H, H_{Ph}), 5.52 (s, 1 H, PhCH), 5.2 (m, 2 H, H_3, CHOCO), 4.53 (s, 2 H, $PhCH_2O$), 4.27 (dt, 1 H, H_{6e}, $J_{6e,6a}$ = 10, $J_{6e,5}$ = 5), 4.15 (dd, 1 H, H_2, $J_{2,3}$ = 10, $J_{2,1}$ = 3.5), 3.52 (ddd, 1 H, H_5, $J_{5,6e}$ = $J_{5,4}$ = 5, $J_{5,6a}$ = 9.5), 0.9 (t, 9 H, 3 × CH_3); $[\alpha]^{20}_D$ −27.5° (*c* 10, $CHCl_3$); MS *m/e* 1062 $[MH]^+$, 972 $[M - PhCH_2]^+$, 757. Anal. Calcd $C_{64}H_{103}NO_{11}$ (1062.53): C, 72.35; H, 9.77; N, 1.32. Found: C, 71.18; H, 8.69; N, 1.01.

[4,6-*O*-Benzylidene-2-[(*R*)-3-(benzyloxy)tetradecanamido]-2-deoxy-α-D-glucopyranosyl]acetic acid (16c) was obtained in 15% yield from **14d** by using the method described for **13a** and chromatography with chloroform–methanol 9:1: TLC R_f = 0.5 (chloroform–methanol 7:1); NMR ($CDCl_3$) δ 7.5–7.2 (m, 10 H, H_{Ph}), 5.55 (s, 1 H, PhCH), 4.76 (m, 1 H, H_1), 4.54 (s, 2 H, $PhCH_2O$), 0.80 (t, 3 H, CH_3); MS *m/e* 626 $[MH]^+$, 528 $[M - PhCO]^+$. Anal. $C_{36}H_{51}NO_8$ (625.81) C, H, N.

[2-Deoxy-2-[(*R*)-3-hydroxytetradecanamido]-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-α-D-glucopyranosyl]acetic acid (17a). A solution of **15a** (2.237 g, 2.17 mmol) and 10% Pd/C (1.0 g) in a mixture of tetrahydrofuran–water (10:1, 440 mL) was hydro-

genolyzed at room temperature for 15 h. The mixture was filtered, evaporated, and chromatographed on LH-20 with methanol yielding 1.34 g (92%): TLC R_f = 0.7 (chloroform–methanol–water–acetic acid 80:25:5:5); NMR ($CDCl_3$–CD_3OD 3:1) δ 5.02 (dd, 1 H, H_3, $J_{3,2}$ = 8.75, $J_{3,4}$ = 9.75), 4.55 (m, 1 H, H_1), 4.23 (dd, 1 H, H_2, $J_{2,1}$ = 5, $J_{2,3}$ = 8.75), 4.0–3.6 (m, 6 H, 2 × CHOH, H_{6e}, H_{6a}, H_5, H_4), 0.88 (t, 6 H, 2 × CH_3); $[\alpha]^{20}_D$ +15.3° (c 10, $CHCl_3$–CH_3OH 1:1); MS m/e 674 $[MH]^+$, 592, 520, 449 [M – MyrOH]$^+$, 209. Anal. $C_{36}H_{67}NO_{10}$ (673.94) C, H, N.

[2-Deoxy-2-[(*R*)-3-hydroxytetradecanamido]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranosyl]acetic Acid (17b). Hydrogenolysis of **15b** and **16a** and purification were carried out as described for **17a**, affording **17b** in 92% yield: TLC R_f = 0.35 (chloroform–methanol–water–acetic acid 80:8:1:1); NMR ($CDCl_3$–CD_3OD 1:1) δ 5.42 (dt, 1 H, CHOCO), 5.02 (dd, 1 H, H_3, $J_{3,2}$ = 9, $J_{3,4}$ = 8), 4.6 (ddd, 1 H, H_1, $J_{1,2}$ = $J_{1,1A}$ = 5, $J_{1,1B}$ = 10), 4.22 (dd, 1 H, H_2, $J_{1,2}$ = 5, $J_{2,3}$ = 9), 3.95 (m, 1 H, CHOH), 3.8–3.62 (m, 3 H, H_{6e}, H_{6a}, H_5), 3.60 (t, 1 H, H_4, $J_{4,5}$ = 9), 0.89 (t, 9 H, 3 × CH_3); $[\alpha]^{20}_D$ (Tris salt) +13.8° (c 10, $CHCl_3$–CH_3OH 1:1); MS m/e 884 $[MH]^+$, 448 [M – MyrOMyr]$^+$. Anal. $C_{50}H_{93}NO_{11}$ (884.30) C, H, N.

[2-Acetamido-2-deoxy-2-[(*R*)-3-hydroxytetradecanamido]-α-D-glucopyranosyl]acetic Acid (17c). Hydrogenolysis of **15c** and purification were performed as described for **17a**, yielding **17c** in 88% yield: TLC R_f = 0.5 (chloroform–methanol–water–acetic acid 80:25:5:5); NMR ($CDCl_3$–CD_3OD 3:1) δ 5.0 (dd, 1 H, H_3, $J_{3,4}$ = 7, $J_{3,2}$ = 9), 4.54 (dt, 1 H, H_1, $J_{1,2}$ = $J_{1,1A}$ = 5, $J_{1,1B}$ = 9.5), 4.23 (dd, 1 H, H_2, $J_{1,2}$ = 5, $J_{2,3}$ = 9), 4.0 (m, 1 H, CHOH), 3.9–3.6 (m, 4 H, H_{6e}, H_5, H_{6a}, H_4), 1.96 (s, 3 H, CH_3CON), 0.88 (t, 3 H, CH_3); MS m/e 490 $[MH]^+$, 440 [M – CH_3CO]$^+$, 264 [M – MyrOH]$^+$, 246 [264 – H_2O], 204 [246 – CH_3CO]. Anal. $C_{24}H_{43}NO_9$ (489.61) C, H, N.

[2-Deoxy-2-[(*R*)-3-hydroxytetradecanamido]-α-D-glucopyranosyl]acetic Acid (17d). Hydrogenolysis of **15b** and **16a** and purification were performed as described for **17a**, yielding **17d** in 92% yield: TLC R_f = 0.27 (chloroform–methanol–water–acetic acid 80:25:5:5); NMR ($CDCl_3$–CD_3OD 1:1) δ 3.95 (m, 2 H, H_2, HCHO), 3.8 (m, 2 H), 0.89 (t, 3 H, CH_3); $[\alpha]^{20}_D$ +21.9° (c 10, $CHCl_3$–CH_3OH 1:1); MS m/e 470 $[MNa]^+$, 448 $[MH]^+$, 222 [MH – MyrOH]. Anal. $C_{22}H_{41}NO_8$ (447.57) C, H, N.

[2-Deoxy-2-[(*R*)-3-hydroxytetradecanamido]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]acetic acid (17e) was prepared by hydrogenolysis of **16b** as described above in 62% yield: TLC R_f = 0.23 (chloroform–methanol–water–acetic acid 80:8:1:1); NMR ($CDCl_3$–CD_3OD 1:1) δ 5.19 (m, 1 H, CHOCO), 4.96 (dd, 1 H, H_3, $J_{3,2}$ = 10.5, $J_{3,4}$ = 9.5), 4.0–3.5 (m, 7 H, H_1, H_2, H_4, H_5, H_{6a}, H_{6e}, CHOH), 0.89 (t, 9 H, 3 × CH_3); $[\alpha]^{20}_D$ –12.4° (c 10, $CHCl_3$–CH_3OH 1:1); MS m/e 884.5 $[MH]^+$, 448 [M – MyrOMyr]. Anal. $C_{50}H_{93}NO_{11}$ (884.30) C, H, N.

Benzyl [2-[(*R*)-3-(Benzyloxy)tetradecanamido]-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α,β-D-glucopyranosyl]acetate (18a). A sample of **10d** (6.68 g, 10 mmol) was acylated with (*R*)-3-(tetradecanoyloxy)tetradecanoic acid as described for the preparation of compounds **15**. Chromatography with toluene–ethyl acetate 9:1 afforded 7.96 g (72%) of a white solid. TLC R_f = 0.6 (toluene–ethyl acetate 9:1); NMR ($CDCl_3$) δ 7.3 (m, 10 H, H_{Ph}), 6.42 and 6.11 (2 × d, 1 H (α:β 5:1), $J_{NH,2(\alpha)}$ = 7.25, $J_{NH,2(\beta)}$ = 10), 5.15 (m, 1 H, HCOCO), 5.08 (s, 2 H, $PhCH_2O$), 495 (dd, 1 H, H_3, $J_{3,2}$ = 11, $J_{3,4}$ = 9), 4.8 (m, 0.8 H, $H_{1\alpha}$), 4.56 and 4.44 (AB q, 2 H, $PhCH_2O$, J_{AB} = 12), 4.31 (m, 1 H, H_2), 3.8 (dt, 1 H, CHOBn), 1.45 (s, 3 H, CH_3), 1.35 (s, 3 H, CH_3), 0.89 (t, 9 H, 3 × CH_3); $[\alpha]^{20}_D$ +10.1° (c 10, CH_2Cl_2); MS m/e 1104 $[MH]^+$, 1046, 996 [M – $PhCH_2O$]$^+$, 209. Anal. $C_{67}H_{109}NO_{11}$ (1104.62) C, H, N.

Benzyl [2-[(*R*)-3-(benzyloxy)tetradecanamido]-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-4,6-*O*-isopropylidene-α,β-D-glucopyranosyl]acetate (18b) was prepared by acylation of **10d** (9.0 g, 13.5 mmol) with (*R*)-3-(benzyloxy)-tetradecanoic acid[49] using the procedure described above. Chromatography with toluene–ethyl acetate (2:1) gave 12.16 g (92%) of a white solid: TLC R_f = 0.5 (toluene–ethyl acetate 1:1); NMR ($CDCl_3$) (5:1 mixture of anomers) δ 7.3 (m, 15 H, H_{Ph}), 6.33, 6.06 (2 × d (5:1), 1 H, NH, $J_{NH,2(\alpha\text{-anom})}$ = 7.25, $J_{NH,2(\beta\text{-anom})}$ = 10), 5.08 (s, 2 H, $PhCH_2CO$), 5.0 (dd, 1 H, H_3, $J_{3,2}$ = 11.25, $J_{3,4}$ = 3.5), 4.75 (m, 0.8 H, $H_{1\beta}$), 4.6–4.3 (m, 5 H, 2 × $PhCH_2$, H_2), 1.4 and 1.3 (2 × s, 2 × CH_3), 0.88 (t, 6 H, 2 × CH_3); $[\alpha]^{20}_D$ +23.6° (c 10, CH_2Cl_2–CH_3OH 5:1). Anal. $C_{60}H_{89}NO_{10}$ (984.38) C, H, N.

Benzyl [2-[(*R*)-3-(Benzyloxy)tetradecanamido]-2-deoxy-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-glucopyranosyl]acetate (19a and 19b). The anomeric mixture **18a** (7.96 g, 7.2 mmol) in dichloromethane (750 mL) at ambient temperature was treated with *p*-toluenesulfonic acid (1.0 g). After 1 h the solvent was evaporated and the residue chromatographed with toluene–ethyl acetate (gradient from 4:1 to 1:1) to afford 5.55 g (72%) of the α-anomer **19a** as a white solid and 682 mg (9%) of the β-anomer **19b** as a white solid.

α-Anomer 19a: TLC R_f = 0.56 (toluene–ethyl acetate 1:1); NMR ($CDCl_3$) δ 7.3 (m, 10 H, H_{Ph}), 6.81 (d, 1 H, NH, $J_{NH,2}$ = 8), 5.2 (m, 3 H, HCOCO, $PhCH_2OCO$), 4.84 (dd, 1 H, H_3, $J_{3,2}$ = 9, $J_{3,4}$ = 8), 4.65 (dt, 1 H, H_1, $J_{1,2}$ = $J_{1,1A}$ = 5, $J_{1,1B}$ = 11), 4.52 (AB, 2 H, $PhCH_2O$, J_{AB} = 11), 4.17 (dt, 1 H, H_2, $J_{2,1}$ = 5, $J_{2,3}$ = 8), 0.88 (t, 9 H, 3 × CH_3); $[\alpha]^{20}_D$ +17.8° (c 10, CH_2Cl_2); MS m/e 1064 $[MH]^+$, 957 [M – $PhCH_2O$]$^+$, 836, 610, 520, 209. Anal. $C_{64}H_{105}NO_{11}$ (1064.49) C, H, N.

β-Anomer 19b: TLC R_f = 0.5 (toluene–ethyl acetate 1:1); NMR ($CDCl_3$) δ 7.3 (m, 10 H, H_{Ph}), 6.24 (d, 1 H, NH, $J_{NH,2}$ = 9.5), 5.1 (m, 1 H, CHOCO), 5.18 (s, 2 H, $PhCH_2CO$), 4.78 (dd, 1 H, H_3, $J_{2,3}$ = 10, $J_{3,4}$ = 9), 4.57, 4.42 (AB, 2 H, $PhCH_2O$, J_{AB} = 11), 3.96 (q, 1 H, H_2, $J_{2,1}$ = $J_{2,3}$ = $J_{2,NH}$ = 9.5), 0.88 (t, 9 H, 3 × CH_3); $[\alpha]^{20}_D$ –8.0° (c 10, CH_2Cl_2); MS m/e 1064 $[MH]^+$, 974, 956 [M – $PhCH_2O$]$^+$, 838, 746, 610, 520. Anal. $C_{64}H_{105}NO_{11}$ (1064.49) C, H, N.

Benzyl [2-[(*R*)-3-(Benzyloxy)tetradecanamido]-3-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-D-glucopyranosyl]acetate (19c and 19d). The anomeric mixture **18b** (12.16 g, 12.35 mmol) was deprotected as described for **19a,b**. Chromatography with toluene–ethyl acetate 7:4 afforded 7.11 g (61%) of the α-anomer **19c** as a white solid followed by 1.98 g (17%) of the β-anomer **19d** (white solid).

α-Anomer 19c: TLC R_f = 0.49 (toluene–ethyl acetate 1:1); NMR ($CDCl_3$) δ 7.3 (m, 15 H, H_{Ph}), 6.78 (d, 1 H, NH, $J_{NH,2}$ = 9), 5.13, 5.07 (AB, 2 H, $PhCH_2OCO$, J_{AB} = 5.5), 4.86 (dd, 1 H, H_3, $J_{3,4}$ = 7, $J_{3,2}$ = 7.5), 4.53 (m, 5 H, 2 × $PhCH_2O$, H_1), 4.17 (ddd, 1 H, H_2, J = 8, J = 5), 0.88 (t, 6 H, 2 × CH_3); $[\alpha]^{20}_D$ +13.4° (c 10, $CHCl_3$); MS m/e 944 $[MH]^+$, 854 [M – $PhCH_2$]$^+$, 610 [M – MyrOBn]$^+$, 628 [M – MyrOBn at pos 2], 520. Anal. $C_{57}H_{85}NO_{10}$ (944.31) C, H, N.

β-Anomer 19d: TLC R_f = 0.24 (toluene–ethyl acetate 1:1); NMR ($CDCl_3$) δ 7.3 (m, 15 H, H_{Ph}), 6.14 (d, 1 H, NH, $J_{NH,2}$ = 9.5), 5.09 (s, 2 H, $PhCH_2OCO$), 4.57 and 4.40 (AB, 2 H, $PhCH_2O$, J_{AB} = 11), 4.51 (s, 2 H, $PhCH_2O$), 3.96 (q, 1 H, H_2, J = 9), 0.89 (t, 6 H, 2 × CH_3); $[\alpha]^{20}_D$ –21.7° (c 10, $CHCl_3$); MS m/s 944 $[MH]^+$, 854 [M – Bn], 628 [M – MyrOBn], 610 [628 – H_2O], 520 [628 – Bn – H_2O]. Anal. $C_{57}H_{85}NO_{10}$ (944.3) C, H, N.

Benzyl [2-[(*R*)-3-(Benzyloxy)tetradecanamido]-3-*O*-[(R)-3-(benzyloxy)tetradecanoyl]-2-deoxy-6-*O*-(triphenylmethyl)-α-D-glucopyranosyl]acetate (20). A solution of **19c** (1.79 g, 1.9 mmol) in dry pyridine (50 mL) at ambient temperature was treated with chlorotriphenylmethane (1.06 g, 3.8 mmol) and molecular sieve (4 Å, 10.0 g). The mixture was stirred for 20 h, filtered, and evaporated. The residue was chromatographed (toluene–ethyl acetate 7:1), giving 1.49 g (66%) of a white solid: TLC R_f = 0.49 (toluene–ethyl acetate 4:1); NMR ($CDCl_3$) δ 7.4–7.2 (m, 30 H, H_{Ph}), 6.46 (d, 1 H, NH, $J_{NH,2}$ = 7.5), 5.07, 5.0 (AB, 2 H, $PhCH_2OCO$, J_{AB} = 3), 4.91 (dd, 1 H, H_3, $J_{3,2}$ = 10, $J_{3,4}$ = 7.5), 4.63 (ddd, 1 H, H_1, $J_{1,2}$ = $J_{1,1A}$ = 5, $J_{1,1B}$ = 11), 4.5 (m, 4 H, 2 × $PhCH_2O$), 4.25 (ddd, 1 H, H_2, $J_{2,NH}$ = 7.5, $J_{2,3}$ = 10, $J_{2,1}$ = 5), 3.23 (m, 2 H, H_{6a}, H_{6e}), 0.88 (t, 6 H, 2 × CH_3); $[\alpha]^{20}_D$ +22.7° (c 10, CH_2Cl_2); MS m/e 1186 $[MH]^+$, 944 [M – Ph_3C]$^+$, 926 [M – Ph_3CO]$^+$, 836 [926 – $PhCH_2O$]$^+$, 610, 333, 243 [trityl]. Anal. $C_{76}H_{99}NO_{10}$ (1186.64) C, H, N.

Benzyl [2-[(*R*)-3-(benzyloxy)tetradecanamido]-2-deoxy-3,4-*O*-bis[(*R*)-3-(benzyloxy)tetradecanoyl]-6-*O*-(triphenylmethyl)-α-D-glucopyranosyl]acetate (21) was prepared by acylation of **20** (1.38 g, 1.16 mmol) with (*R*)-3-(benzyloxy)-tetradecanoic acid as described for compounds **15**. Chromatography with cyclohexane–ethyl acetate (6:1) gave 700 mg (40%)

(49) Inage, M.; Chaki, H.; Imoto, M.; Shimamoto, T.; Kusumoto, S.; Shiba, T. *Tetrahedron Lett.* **1983**, 2011.

of a white solid. TLC R_f = 0.85 (toluene–ethyl acetate 4:1); NMR ($CDCl_3$) δ 7.3 (m, 35 H, H_{Ph}), 6.68 (d, 1 H, NH, $J_{NH,2}$ = 9.5), 5.08 and 4.98 (AB q, 1 H, $PhCH_2OCO$, J_{AB} = 12), 4.95 (m, 2 H, H_3, H_4), 4.45 (m, 7 H, H_1, 3 × $PhCH_2O$), 4.22 (m, 1 H, H_2), 4.0 (m, 1 H, H_5), 0.89 (t, 9 H, 3 × CH_3); $[\alpha]^{20}_D$ +6.5° (c 10, CH_2Cl_2); MS m/e 1501 $[M]^+$, 1395 $[M - PhCH_2OH]^+$, 1260 $[M - Ph_3C]^+$, 1243 $[M - Ph_3CO]^+$, 1160, 926, 243 [trityl]. Anal. $C_{97}H_{131}NO_{12}$ (1503.13) C, H, N.

Benzyl [2-[(*R*)-3-(Benzyloxy)tetradecanamido]-2-deoxy-3,4-*O*-bis[(*R*)-3-(benzyloxy)tetradecanoyl]-α-D-glucopyranosyl]acetate (22). Trityl ether 21 (700 mg, 0.47 mmol) in dichloromethane (30 mL) at room temperature was treated with *p*-toluenesulfonic acid (20 mg). After 18 h the mixture was evaporated and chromatographed (toluene–ethyl acetate 9:1) to afford 460 mg (78%) of a white solid: TLC R_f = 0.48 (toluene–ethyl acetate 4:1); NMR ($CDCl_3$) 7.3 (m, 20, H_{Ph}), 6.92 (d, 1 H, NH, $J_{NH,2}$ = 10), 5.17, 5.09 (AB, 2 H, $PhCH_2OCO$, J_{AB} = 6), 4.92 (t, 1 H, H_3, J = 4), 4.66 (dd, 1 H, H_4, J = 3, J = 4), 4.47 (m, 7 H, 3 × $PhCH_2O$, H_1), 4.2–4.05 (m, 2 H, H_6), 0.89 (t, 9 H, 3 × CH_3); $[\alpha]^{20}_D$ +1.3° (c 10, CH_2Cl_2); MS m/e 1260 $[MH]^+$, 1170, 1152, 1062, 926, 836. Anal. $C_{88}H_{117}NO_{12}$ (1260.80) C, H, N.

[2-Deoxy-3,4-*O*-bis[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanamido]-α-D-glucopyranosyl]acetic acid (23) was obtained by catalytic hydrogenolysis of **22** (1.22 g, 0.97 mmol) as described for compounds **19**. Crystallization of the crude product (980 mg) from methanol afforded 620 mg (71%) of a white waxy solid: mp 150–151 °C; TLC R_f = 0.26 (chloroform–methanol–water–acetic acid 80:8:1:1; NMR ($CDCl_3$–CD_3OD 1:1) δ 5.15 (dd, 1 H, H_3, $J_{3,2}$ = 7.5, $J_{3,4}$ = 6.5), 4.96 (t, 1 H, H_4, J = 6.5), 4.6 (m, 1 H, H_1), 4.3 (dd, 1 H, H_2, $J_{2,3}$ = 7.5, $J_{2,1}$ = 4.5), 0.90 (t, 9 H, 3 × CH_3); $[\alpha]^{20}_D$ +9.8° (c 10, $CHCl_3$–CH_3OH 1:1); MS m/e 900 $[MH]^+$, 674 $[M - MyrOH]^+$, 658 $[M - MyrOH - NH]^+$. Anal. $C_{50}H_{93}NO_{12}$ (900.30) C, H, N.

Preparation of the Disaccharides. Preparation of the Buffer Solutions Used. One liter of *buffer solution A* contained Tris Hydrochloride (12.1 g, 100 mmol), NaCl (234 g, 4000 mmol), ethylenediaminetetraacetic acid (EDTA; 750 mg, 2 mmol), dithioerythritol (DTE) (308 mg, 2 mmol), Triton X-100 (5 mL, 0.5%), set to pH 7 with HCl.

One liter of *buffer solution B* contained Tris hydrochloride (1.21 g, 10 mmol), EDTA (7.44 mg, 0.2 mmol), DTE (30.8 mg, 0.2 mmol), pH 7.

Preparation of Lipid A Synthase. Fermentation broth was inoculated in a 55-L Giovanola 70C fermenter (medium: LB broth, 1% tryptone, 0.5% yeast extract, 1% NaCl, 1% fructose, 50 mM phosphate buffer (pH 7), 50 mg/L ampicillin, 0.1 mL/L Glanapon 2000) at 30 °C with 2% of a culture of *E. coli* JB1104 (Δ2514) grown overnight in the LB broth. The fermentation was performed with the following parameters: starting OD_{576} = 0.095, stirring 1000 rpm, air rate 40 L/min, pO_2 = 40% of saturation, temperature 30 °C. The pH was not regulated and decreased to 6.6. After 3 h of fermentation (OD_{576} = 0.70) 10 g/L fructose and 5 g/L arabinose were added at once. Subsequently 270 mL/h of a solution of 187.5 g/L arabinose and 137.5 g/L fructose was added continuously for 12 h (end OD_{576} = 6.40). The cells were harvested by centrifugation (8500*g*/10 min), washed, resuspended in buffer solution B, and disrupted by ultrasonication (~100 W/5 min). After centrifugation (30000*g*/4 °C/20 min) and ultracentrifugation (100000*g*/4 °C/90 min), a fractionated $(NH_4)_2SO_4$ precipitation followed. Finally, the pellets of 0–20% and 20–40% $(NH_4)_2SO_4$ saturation were discarded, and the pellet of the 70% $(NH_4)_2SO_4$ saturation was resuspended in buffer solution B. This enzyme preparation was ready to use and could be stored at −20 °C for several months without losing catalytic activity.

[2-Deoxy-2-acetamido-6-[2′-deoxy-2′-[(*R*)-2′-hydroxytetradecanamido]-3′-*O*-[(*R*)-hydroxytetradecanoyl]-α-D-glucopyranosyl]-3-[(*R*)-3-hydroxytetradecanoyl]-α-D-glucopyranosyl]acetic acid (25a) was prepared by using the procedure outlined below for **25b**: yield 50%, TLC R_f = 0.75 (chloroform–methanol–acetic acid–water 25:15:2:4), NMR (free acid) ($CDCl_3$–CD_3OD 3:1) δ 4.97 (t, 2 H, H_3, $H_{3'}$), 4.60 (d, 1 H, $H_{1'}$), 4.55 (m, 1 H, H_1), 4.25 (dd, 1 H, H_2), 3.6 (bq, 2 H, H_4, $H_{4'}$), 2.8–2.2 (m, 8 H, $-CH_2CO-$), 1.96 (s, 3 H, CH_3CON), 0.89 (t, 9 H, 3 × CH_3); MS m/e 1103 $[MH]^+$, 614 base peak [M − *C*-glycosidic sugar]. Anal. $C_{58}H_{106}N_2O_{17}$ (1103.49) C, H, N.

[2-Deoxy-6-[2′-deoxy-2′-[(*R*)-3-hydroxytetradecanamido]-3′-*O*-[(*R*)-hydroxytetradecanoyl]-α-D-glucopyranosyl]-2-[(*R*)-3-hydroxytetradecanamido]-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-α-D-glucopyranosyl]acetic Acid (25b). A solution of **17a** (200 mg, 0.252 mmol) and UDP-2-deoxy-3-*O*-[3(*R*)-hydroxytetradecanoyl]-2-[3(*R*)-hydroxytetradecanoylamido]-α-D-glucopyranose (265 mg, 0.252 mmol) in buffer solution B (100 mL) was diluted with buffer solution A (50 mL) and then treated with the lipid A synthase preparation (100 mL). The mixture was incubated at 30 °C. After 6 days the reaction was complete (TLC). For workup and purification the following chromatography procedure proved to be the most efficient: RP-18 silica gel (50 g) was washed with freshly distilled pyridine. Pyridine (250 mL) and the reaction mixture were added under cooling slowly in that order. The mixture was degassed by careful, slight evacuation (foaming!) and shaken for 15 min. More RP-18 (50 g) was washed with pyridine and used as column filling. The RP-18/reaction mixture suspension was added subsequently to the column and eluated with a pyridine–acetic acid–water mixture (gradient 39:1:60 to 70:1:29, to wash out the salts, and then to 98:1:1, where the disaccharide was eluted). The combined product containing fractions were freeze-dried and the procedure repeated (gradient 70:1:29 to 98:1:1). Finally the residue was suspended in ether and filtered and the filtrate evaporated under vacuum, affording 190 mg (58.5 %) of a white solid. In order to prepare the lysine salt the residue was suspended in pyrogen-free water (100 mL) and an equimolar amount of L-lysine (free base) was added as a 100 mM solution in water. Freeze-drying afforded the mono(L-lysine) salt of the disaccharide. TLC R_f = 0.67 (chloroform–methanol–acetic acid–water 25:15:2:4); NMR (free acid) of ($CDCl_3$–CD_3OD 3:1) 5.08–4.92 (m, 2 H, H_3, $H_{3'}$), 4.59 (d, 1 H, $H_{1'}$), 4.55 (m, 1 H, H_1), 4.25 (ddd, 1 H, H_2), 4.1–3.7 (m, 11 H), 3.59 (bq, 1 H, H_4, $H_{4'}$), 2.8–2.12 (m, 10 H, $-CH_2CO-$), 0.87 (t, 12 H, 4 × CH_3); MS m/e 1287 $[MH]^+$, 1061 [M − 1 fatty acid], 835 [M − 2 fatty acids], 614 base peak [M − *C*-glycosidic sugar]. Anal. $C_{70}H_{130}N_2O_{18}$ (1287.80) C, H, N.

Biology. Enhancement of **Nonspecific resistance (NSR)** was evaluated by treating $B_6D_2F_1$ mice subcutaneously with cyclophosphamide (Endoxan) (200 mg/kg) 4 days before infection (day −4). On day −1, the test groups were treated intraperitoneally with the test compounds (12.5 mg/kg) and one group was treated intraperitoneally with LPS (*S. abortus equi*, Sigma Chemical Co., 250 mg/kg) to be used as control. Finally, the mice were infected on day 0 by intravenous inoculation with *P. aeruginosa* (Δ12), inoculum = 6 × 10 *cfu* (LD_{95}). The table shows the survival rate after an observation period of 4 days. The buffer-treated and infected control mice usually died within 48 h after infection. For compounds inducing a high survival rate the ED_{50} was determined and calculated using Probit[47] analysis.

Endotoxin (ET) Shock Induction. Lethal toxicity in galactosamine sensitized mice was tested by the method of Galanos.[46] Serial dilutions of test compounds were administered iv to groups of six mice (C57BL/6) via the tail vein in pyrogen-free saline solution (total volume administered 0.2 mL). Simultaneously, galactosamine (400 mg/kg) was administered intraperitoneally to each mouse. The LD_{50} was calculated from the survivors 1 day later using Probit[47] analysis.

Acknowledgment. We thank Gerald Ehn for the fermentation of lipid A synthase, Michael Kern, Karl Bednarik, and Evelyne Janzek for technical assistance and Dr. Gerhard Schulz for recording and interpretation of NMR spectra. We are most grateful to Dr. Hans Loibner and Dr. Peter Stütz for numerous discussions.