Acyclic Analogues of Lipid A: Synthesis and Biological Activities

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The synthesis of a series of novel acyclic analogues of lipid A, the lipophilic terminal of lipopolysaccharide (LPS), is reported. In these compounds, the reducing glucose unit of lipid A has been replaced by an acyclic analogue unit (abbreviated as AAU) consisting of a spacer (of varying length), an (*R*)-3-hydroxytetradecanamido moiety (of varying configuration at the carbon of attachment), and a CO_2H group. The AAU has been attached to the anomeric carbon of the nonreducing glucose unit of lipid A, either through glycosidic linkage or through an acyl linkage. Further, amide isosteres of these acyclic analogues have been prepared using suitably protected 2,3-diamino-2,3-dideoxyglucose instead of 2-amino-2-deoxyglucose. All the compounds were well characterized and were tested for their ability to induce TNF-α in mouse bone marrow-derived macrophages, to enhance nonspecific resistance to infection in mice and to induce endotoxic shock in mice. The results showed a dramatic dependence, for the first time, on the length of the spacer and on the configuration of the carbon bearing the amido group in the AAU part of the analogues.

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 (Chart I), which is a β-(1→6)-glucosamine disaccharide acylated with several fatty acids—usually (*R*)-3-hydroxytetradecanoic acid—and bearing two phosphate groups in positions 1 and 4′. 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.[10-23] Compounds

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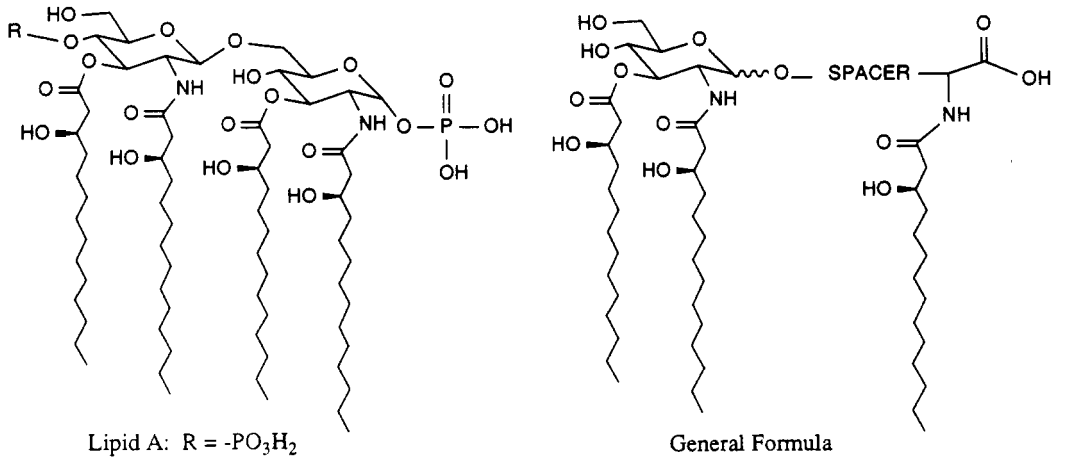
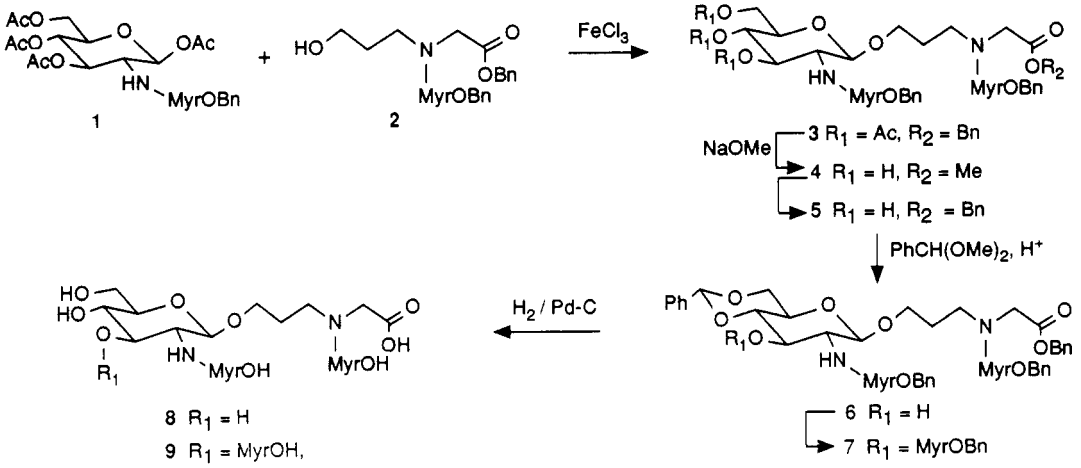
Chart I



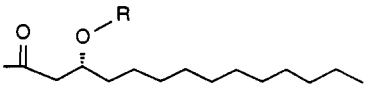
Scheme I. Synthesis of Lipid A Analogues Incorporating Glycosidic Linkage



isolated from chemically manipulated or degraded LPS 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 total synthesis of chemically defined analogues of lipid A and its partial structures.

From what is known so far regarding the structure–activity relationships of lipid A and lipid X analogues, the following general conclusions can be drawn: (1) The presence of three long-chain fatty acids per molecule is a prerequisite for immunostimulatory activity.[22-25] (2) The disaccharide structures are, in general, more active than the monosaccharide structures. (3) The phosphate group can be replaced by the isosteric group CH_2COOH, without loss of activity.[23] The disaccharide structures, despite their higher activity, can be prepared only through prohibitively long synthetic sequences. Our objective, therefore, was to design synthetically simpler analogues of lipid A of the general formula shown in Chart I, and to study their structure–activity relationships.

Chart II. Abbreviations Used in the Reaction Schemes



Results

Chemistry. Preparation of the Lipid A Analogues 8 and 9 Incorporating Glycosidic Linkage. The β-acetate **1** was glycosylated with the alcohol **2** using the $FeCl_3$ method[26] to afford the β-glycoside **3** (Scheme I).

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Scheme II. Synthesis of Lipid A Analogues Incorporating Anomeric Acyl Linkage

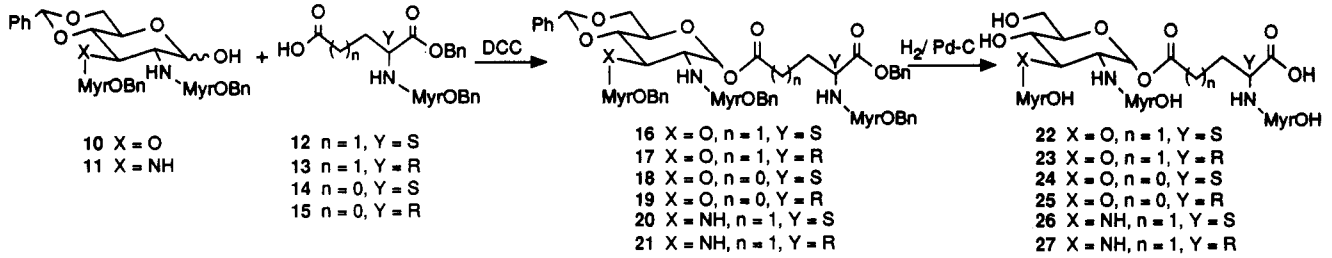

Table I. Biological Activities of Lipid A Analogues[a]

entry	NSR[b] survival rate	NSR ED_{50}, mg/kg	TNF-α, IU/mL −IFN_γ[c]	TNF-α, IU/mL +IFN_γ[d]	ET shock[e] LD_{50}, mg/kg
8	3/10	ND[f]	00	00	>5.0
9	10/10	1.5	1498 (523)	3050 (920)	0.1
22	9/10	5.7	576 (270)	3285 (1511)	0.3
23	10/10	0.3	688 (518)	>2409 (>2409)	0.04
24	8/10	>12.5	446 (0)	899 (25)	1.0
25	10/10	3.1	618 (218)	2768 (2708)	<0.05
26	8/10	1.5	1069 (175)	1570 (294)	0.2
27	3/10	>25	464 (27)	609 (151)	5.1
LPS	10/10	1.9×10^{-3}	1273 (916)	>4138 (1944)	2×10^{-5}

[a] All in vivo tests are carried out by iv administration. All the compounds are tested as Tris salts. [b] NSR (nonspecific resistance) against a *Pseudomonas aeruginosa* (Δ12) infection: administration of a single dose (12.5 mg/kg) 1 day prior to infection, group size 10 mice. [c] TNF-α IU/mL induced in the absence of IFN^γ by the compounds (8, 9, 22–27) at a concentration of 10 μg/mL (in parentheses: at a concentration of 1 μg/mL), and by LPS (last entry) at a concentration of 10 ng/mL (in parentheses: at a concentration of 1 ng/mL). [d] TNF-α 100 IU/mL induced after preincubation with IFN_γ, by the compounds (8, 9, 22–27) at a concentration of 10 μg/mL (in parentheses: at a concentration of 1 μg/mL) and by LPS (last entry) at a concentration of 10 ng/mL (in parentheses: at a concentration of ng/mL). [e] Endotoxin (ET) shock induction, simultaneous iv administration of substance and galactosamine (400 mg/kg). [f] Not determined.

Only small amounts of the corresponding α-glycoside could be detected in the reaction mixture (^{1}H-NMR). Zemplen's deacetylation of **3** using NaOMe/MeOH afforded the methyl ester **4**. The ester **4** was saponified and acidified, and the cesium salt of the acid was treated with benzyl bromide to give the benzyl ester **5**, which upon benzylidenation afforded **6**. Hydrogenolytic deprotection of **6** led to **8**. On the other hand, acylation of **6** with (*R*)-3-(benzyloxy)tetradecanoic acid (HO-MyrOBn) using DCC/DMAP led to **7** which after hydrogenolytic deprotection afforded **9**.

Preparation of the Lipid A Analogues 22–27 Incorporating Anomeric Acyl Linkage. The amino acid derivatives **12–15** were prepared through N-acylation of the corresponding amino acid α-benzyl esters with the *N*-hydroxysuccinimide ester of HO-MyrOBn in good yields (Scheme II). The protected glucose derivatives **10**[27] and **11**[28] were then coupled with **12–15** using DCC/DMAP, giving **16–21** in moderate yields. The corresponding β-acyl derivatives (^{1}H-NMR) were formed only in low yields, and no attempt was made to isolate and characterize them fully. Hydrogenolytic deprotection of **16–21** resulted in the lipid A analogues **22–27** in excellent yields.

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[29,30] ("nonspecific resistance", NSR) after iv 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 ip administration of galactosamine.[31] Furthermore, the lipid A analogues and LPS were investigated for their potency to induce TNF-α in murine macrophage cultures.[32] TNF-α is one of the most active biological multipotential proinflammatory cytokines induced by LPS playing a central role in endotoxicity.

The biological results for all compounds and for the standard LPS are given in Table I. The second column shows the survival rates of mice after single dose iv application of the respective compounds in the mouse infection model. The third column contains the ED_{50} values determined for all compounds with a survival rate greater than 70%. The fourth column shows the TNF-α values (international units) induced in murine macrophages with

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(27) Compound **10** was prepared through bisacylation of 2-amino-4,6-*O*-benzylidene-2-*N*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxyglucose using (*R*)-3-(benzyloxy)tetradecanoic acid, DCC and DMAP, followed by regioselective deacylation of the resulting product, using 2-aminoethanol in EtOAc.
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or without preactivation by IFN-γ and the fifth shows the LD_{50} values in the endotoxic shock model. The biological data are quite consistent within themselves. Firstly, comparing the biological activities of **8** with those of **9**, **8** (bearing only two fatty acids) is practically inactive and nontoxic, whereas **9** (bearing an additional fatty acid and having otherwise identical structural features) is relatively quite active and also toxic. This demonstrates again, the prerequisite of three fatty acids for biological activity, which was evident in earlier studies.[22-25] By comparison of the activities pairwise (**8** with **9**; **22** with **23**, and so on), the more toxic compound (lower LD_{50}) induces, as expected, more TNF-α in vitro and also is, however, more potent in NSR (lower ED_{50} or higher survival rate). Lack of a direct linear correlation between the LD_{50} or TNF induction and the ED_{50}, indicates the multifaced nature of the immunological factors responsible for NSR. The fact that the β-glycoside analogue **9** is considerably active would mean that the reducing sugar moiety of lipid A could be simplified without losing the activity. Further, the good activities of the non-glycosidic acyl analogues **22–26** clearly demonstrate that even the glycosidic bond is not a prerequisite for the activity. More dramatic is the dependence of the biological activity on the configuration of the α-carbon (of the amino acid moiety) and the length of the spacer (Chart I). Thus, comparing **22** with **23**, and **24** with **25**, the compounds with *R* configuration are considerably more potent (NSR) than the ones with *S* configuration. Comparing **22** with **24**, and **23** with **25**, one can see that the glutamic acid spacer is better than the aspartic acid spacer. It may be mentioned, in this connection, that a similar dependence of activity on stereochemistry and spacer length was also observed with **22–25** in a different assay (in vitro activation of 7OZ/3 pre-B cells).[33] Finally, it is noteworthy that the activities of the amide isosteres **26** and **27** are also dependent on the configuration of the α-carbon of the amino acid moiety, but, in this case the dependence is opposite to that observed with **22–25**. The reason for this, however, is not obvious.

The NSR activities of **9**, **25**, and **26** are comparable with those[23] of other mono- and disaccharide derivatives, including the lipid A precursor (Chart 1). To our knowledge, **23** is the most active total synthetic compound in our NSR model. However, these compounds are relatively more toxic and the therapeutic windows (LD_{50}/ED_{50}) are not favorable when compared with those of other monosaccharide analogues.[34] Further studies are required for reducing the toxicity and retaining the already achieved high activities.

Conclusions

With regard to structure–activity relationships, the following conclusions could be drawn: (1) The presence of three fatty acid moieties is a prerequisite for activity. (2) The glycosidic bond is not a prerequisite for the activity. (3) The reducing glucose unit of lipid A could be replaced by a unit containing a spacer, a fatty acid, and a COOH group; this unit may be attached to the nonreducing glucose unit at the anomeric position either through a glycosidic bond or through an acyl linkage. (4) The length of the spacer and the configuration of the α-carbon are crucial for the activity.

Experimental Section

Chemistry. General Procedures. ^{1}H-NMR spectra were recorded with a Bruker WM-250 spectrometer at 250 MHz (not mentioned in text) or with a Bruker AMX-500 at 500 MHz and are reported only for representative compounds. Chemical shifts are reported in ppm relative to internal Me_4Si. Coupling constants (*J*) are reported in hertz. Unless mentioned, all assignments are based on comparison with literature values. The abbreviations indicating the multiplicity of the signals are s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; overl m's, overlapping multiplets. 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). Solvents were AR grade and were used without purification excepting dichloromethane which was dried over P_2O_5. Pyrogen-free water was used for all final steps. Unless otherwise mentioned, 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: Tris = tris(hydroxymethyl)aminomethane, DCC = dicyclohexylcarbodiimide, DMAP = 4-(dimethylamino)pyridine.

Benzyl 2-[(3-Hydroxypropyl)amino]acetate. A mixture of benzyl bromoacetate (40 mL, 0.25 mol) and dichloromethane (100 mL) was added over a period of 15 min to a mixture of 3-hydroxypropylamine (78 mL, 1.02 mol) and dichloromethane (500 mL) cooled to −60 °C. The mixture was allowed to come to room temperature slowly over a period of 4 h and was washed successively with water (300 mL) and brine (3 × 300 mL), dried ($MgSO_4$), and the solvent removed to give 37 g of crude product. Chromatography using ether removed all impurities. Further elution using dichloromethane–methanol (1:1) gave the title compound as a colorless syrup (19.9 g, 36%), TLC R_f = 0.37 (CH_2Cl_2–MeOH 9:1).

***N*-[[(Benzyloxy)carbonyl]methyl]-*N*-(3-hydroxypropyl)-(*R*)-3-(benzyloxy)tetradecanamide (2).** A mixture of benzyl 2-[(3-hydroxypropyl)amino]acetate (5.0 g, 22 mmol), (*R*)-3-(benzyloxy)tetradecanoic acid *N*-hydroxysuccinimide ester (9.5 g, 22 mmol), *N*-ethyldiisopropylamine (2.8 g, 22 mmol) and dimethylformamide (23 mL) was stirred at room temperature for 24 h. The solvent was removed under high vacuum, and the residue was dissolved in EtOAc and washed successively with water, 1 N HCl, water, aqueous $NaHCO_3$, and brine. The organic phase was dried ($MgSO_4$), the solvent removed, and the residue chromatographed using toluene–EtOAc 25:10 to give **2** (3.6 g, 30%) as a colorless syrup: ^{1}H-NMR ($CDCl_3$, 2:3 mixture of rotamers) δ 0.90 (t, 3 H, Me, *J* = 6.3), 1.20–1.70 (overl m's, 22 H, $C_{11}H_{20}$, $H_2C(CH_2OH)(CH_2N)$), 1.46–1.77 (two br m, 5 H), 2.33 (dd, 0.6 H, CH_2CO, *J* = 15.0, 5.0), 2.42 (dd, 0.4 H, CH_2CO, *J* = 15.0, 5.0), 2.57 (dd, 0.6 H, CH_2CO, *J* = 15.0, 7.5), 2.92 (dd, 0.4 H, CH_2CO, *J* = 15.0, 7.5), 3.39–3.78 (two br m, 4 H, CH_2N, CH_2OH), 3.89 (d, 0.6 H, CH_2CO_2, *J* = 17.5), 3.96–4.14 (two br m, 1 H, HC(OBn)), 3.97 (d, 0.6 H, CH_2CO_2, *J* = 17.5), 4.24 (d, 0.4 H, CH_2CO_2, *J* = 17.5), 4.34 (d, 0.4 H, CH_2CO_2, *J* = 17.5), 4.43–4.57 (ABq, 1.2 H,

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Bn, J = 11.3), 4.55 (s, 0.8 H, Bn), 5.19 (s, 2 H, CO_2Bn), 7.23–7.41 (m, 20 H, Ph); $[\alpha]_D^{20}$ +2.75° (c 0.87, $CHCl_3$); MS m/z 540 (MH^+), calcd 540.37. Anal. ($C_{33}H_{49}NO_5$) C, H, N.

3-[*N*-[[(Benzyloxy)carbonyl]methyl]-*N*-[(*R*)-3-(benzyloxy)tetradecanoyl]amino]propyl 3,4,6-Tri-*O*-acetyl-2-amino-2-*N*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-β-D-glucopyranoside (3). 1,3,4,6-Tetra-*O*-acetyl-2-amino-2-*N*-[(*R*)-3-(benzyloxy)tetradecanoyl)]-2-deoxy-β-D-glucopyranose (1, 2.15 g, 3.24 mmol) was added to a mixture of anhydrous $FeCl_3$ (0.35 g, 2.16 mmol), drierite (1.77 g, 13 mmol), and dry dichloromethane (20 mL) prestirred under argon for 5 min. After stirring for 5 min, a mixture of 2 (0.88 g, 1.6 mmol) and CH_2Cl_2 (5 mL) was added. After stirring for 3 h, again a mixture of 2 (0.88 g 1.6 mmol) and CH_2Cl_2 (5 mL) was added. After 17 h, additional $FeCl_3$ (0.70 g, 4.32 mmol) and 2 (0.35 g, 0.65 mmol) in CH_2Cl_2 (10 mL) were introduced separately into the reaction mixture. After stirring for additional 24 h, the mixture was poured into ice-cold aqueous $NaHCO_3$ under vigorous stirring. The organic phase was separated, and the aqueous phase was extracted with $CHCl_3$. The combined organic phases were stirred with ice–water and the phases separated, and the aqueous phase was reextracted several times with $CHCl_3$. The combined organic phases were dried ($MgSO_4$) and concentrated, and the residue was chromatographed (gradient toluene–EtOAc 5:1 to 2:1) to give 3 (0.81 g, 22% based on 1) as a syrup: ^{1}H-NMR $CDCl_3$, 1:1 mixture of rotamers) δ 0.88 (t, 6 H, CH_3, J = 6.3), 1.18–1.63 (br m, 40 H), 1.97 (s, 1.5 H, Ac), 2.00 (s, 1.5 H, Ac), 2.03 (s, 3 H, Ac), 2.05 (s, 1.5 H, Ac), 2.08 (s, 1.5 H, Ac), 2.22–2.53 (overl m's, 4 H), 2.59–2.76 (m, 1 H), 3.25–3.41 (br m, 2 H), 3.47–3.55 (m, 0.5 H), 3.55–3.65 (m, 0.5 H), 3.67–3.90 (overl m's, 3 H), 3.91–4.61 (overl m's, 11 H), 4.95–5.20 (overl m's, 5 H), 6.39 (d, 1 H, NH, J = 8.8), 7.22–7.42 (m, 15 H, Ph). The ^{1}H-NMR signals of the rotamers coalesced together in DMSO at 373 K. $[\alpha]_D^{20}$ –0.96° (c 1.14, $CHCl_3$); MS m/z 1143 (MH^+), calcd 1143.71. Anal. ($C_{66}H_{98}N_2O_{14}$) C, H, N.

3-[*N*-[(*R*)-3-(Benzyloxy)tetradecanoyl]-*N*-[(methoxycarbonyl)methyl]amino]propyl 2-Amino-2-*N*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-β-D-glucopyranoside (4). A mixture of 3 (1.5 g, 1.3 mmol), MeOH (50 mL), and NaOMe (7 mg, 0.13 mmol) was stirred at room temperature for 22 h. The mixture was cooled in ice bath, acidified to pH 6 with Dowex-H^+ (50 Wx4), and stirred for 5 min, the resin was filtered off, and the filtrate was stripped of the solvent. Chromatography of the residue using ether removed impurities. Further elution with CH_2Cl_2–MeOH 10:1 gave 4 (1.1 g, 89%) as a colorless syrup: $[\alpha]_D^{20}$ –9.35° (c 0.96, $CHCl_3$); MS m/z 941 (MH^+), calcd 941.65. Anal. ($C_{54}H_{88}N_2O_{11}$) C, H, N.

3-[*N*-[(*R*)-3-(Benzyloxy)tetradecanoyl]-*N*-[[(benzyloxy)carbonyl]methyl]amino]propyl 2-Amino-2-*N*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-β-D-glucopyranoside (5). A mixture of 4 (0.91 g, 0.97 mmol), KOH (62 mg, 1.1 mmol), water (1 mL), and MeOH (10 mL) was stirred at room temperature for 4 days. The reaction mixture was diluted with MeOH (50 mL) and water (10 mL), cooled in an ice bath, acidified with Dowex (H^+) to a constant pH (3.6), and stirred for 5 min, and the resin was filtered off and washed with MeOH. The combined filtrate and washings were basified with 20% aqueous Cs_2CO_3 to pH 7.3, the solvent was removed, and the last traces of water were removed through codistillation with MeOH and toluene under vacuum and dried under high vacuum. A mixture of the resulting Cs salt, dimethylformamide (10 mL), and benzyl bromide (187 mg, 1.09 mmol) was stirred at room temperature for 16 h. The solvent was removed under high vacuum, and the residue was taken up in EtOAc, washed with brine, and dried ($MgSO_4$), and the solvent was removed. Chromatography of the residue using EtOAc–MeOH 20:1 gave 5 (0.64 g, 65%) as a colorless syrup: $[\alpha]_D^{20}$ –8.4° (c 0.72, MeOH); MS m/z 1017.6 (MH^+), calcd 1017.68. Anal. ($C_{60}H_{92}N_2O_{11}$) C, H, N.

3-[*N*-[[(Benzyloxy)carbonyl]methyl]-*N*-[(*R*)-3-(benzyloxy)tetradecanoyl]amino]propyl 2-Amino-4,6-*O*-benzylidene-2-*N*-[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-β-D-glucopyranoside (6). A mixture of 5 (0.36 g, 0.35 mmol), 1,1-dimethoxytoluene (162 mg, 1.06 mmol), *p*-toluenesulfonic acid monohydrate (67 mg, 0.35 mmol), and dimethylformamide (8 mL) was rotated for 1.5 h at 75 °C under vacuum (in a rotary evaporator) such that the solvent gently refluxed. Subsequently, the solvent was removed under high vacuum, the residue was taken up in CH_2Cl_2 and washed with aqueous $NaHCO_3$, and the aqueous phase was reextracted with CH_2Cl_2. The combined organic extracts were washed with water and dried ($MgSO_4$), and the solvent was removed. Chromatography of the residue using solvent gradient toluene–EtOAc 3:1 to 18:10 gave 6 (200 mg, 52%) as a colorless semisolid: $[\alpha]_D^{20}$ –14.16° (c 1.34, $CHCl_3$); MS m/z 1105 (MH^+), calcd 1105.71. Anal. ($C_{67}H_{96}N_2O_{11}$) C, H, N.

3-[*N*-[[(Benzyloxy)carbonyl]methyl]-*N*-[(*R*)-3-(benzyloxy)tetradecanoyl]amino]propyl 2-Amino-4,6-*O*-benzylidene-2-*N*,3-*O*-bis[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-β-D-glucopyranoside (7). DCC (52 mg, 0.25 mmol) was added to an ice-cold mixture of 6 (200 mg, 0.18 mmol), (*R*)-3-(benzyloxy)tetradecanoic acid (84 mg, 0.25 mmol), DMAP (10 mg, 0.08 mmol), and CH_2Cl_2 (8 mL). After stirring at 0 °C for 3 h and at room temperature for 4 h, the mixture was diluted with CH_2Cl_2, the precipitate was filtered off, and the filtrate was washed with aqueous $NaHCO_3$ and water. The organic phase was dried ($MgSO_4$) and concentrated under vacuum. Chromatography of the residue using solvent gradient toluene–EtOAc 5:1 to 2:1 gave 7 (218 mg, 85%) as a white powder: ^{1}H-NMR ($CDCl_3$, 1:1 mixture of rotamers) δ 0.88 (t, 9 H, CH_3, J = 6.9), 1.10–1.75 (overl m's, 62 H, $C_{11}H_{20}$, $H_2C(CH_2N)(CH_2O)$), 2.23–2.53 (overl m's, 4 H, CH_2CO), 2.60–2.77 (overl m's, 2 H, CH_2CO), 3.25–3.48 (overl m's, 3 H), 3.51–4.62 (overl m's, 18 H), 5.11–5.30 (overl m's, 3 H, CO_2Bn, H-1), 5.36 (s, 0.5 H, H-C(Ph)(O)(O)), 5.42 (s, 0.5 H, H-C(Ph)(O)(O)), 6.32 (br d, 0.5 H, NH, J = 8.8), 7.16 (br d, 0.5 H, NH, J = 8.8), 7.20–7.42 (m, 25 H, Ph); $[\alpha]_D^{20}$ –22.99° (c 0.96, $CHCl_3$–MeOH 1:1); MS m/z 1421.6 (MH^+), calcd 1421.95. Anal. ($C_{88}H_{128}N_2O_{13}$) C, H, N.

3-[*N*-(Carboxymethyl)-*N*-[(*R*)-3-hydroxytetradecanoyl]amino]propyl 2-Amino-2-deoxy-2-*N*-[(*R*)-3-hydroxytetradecanoyl]-β-D-glucopyranoside (8). A mixture of 6 (207 mg, 0.19 mmol), 10% Pd/C (60 mg), tetrahydrofuran (90 mL), and water (10 mL) was stirred under H_2 atmosphere until completion of the reaction (1 d). The catalyst was filtered off on a bed of Celite, and the filtrate was filtered through a membrane filter (0.02-μm pore size) and the volatiles stripped off under vacuum. The residue was suspended in water and lyophilized. The lyophilizate was washed with ether to leave behind pure 8 (123 mg, 87%): $[\alpha]_D^{20}$ –25.07° (c 0.74, $CHCl_3$–MeOH 1:1); MS m/z 747 (MH^+), calcd for $C_{39}H_{75}N_2O_{11}$ 747.54. Anal. of 8·3H_2O ($C_{39}H_{80}N_2O_{14}$) C, H, N.

3-[*N*-(Carboxymethyl)-*N*-[(*R*)-3-hydroxytetradecanoyl]amino]propyl 2-Amino-2-deoxy-2-*N*,3-*O*-bis[(*R*)-3-hydroxytetradecanoyl]-β-D-glucopyranoside (9) Tris Salt. Prepared analogous to 8. A mixture of the free acid 9 (185 mg, 0.19 mmol), Tris (23 mg, 0.19 mmol), tetrahydrofuran (5 mL), and water (2 mL) was sonicated for 5 min and lyophilized to give the Tris salt (207 mg): ^{1}H-NMR ($CDCl_3$–CD_3OD 1:1) δ 0.89 (t, 9 H, CH_3, J = 6.9), 1.20–1.56 (br m, 60 H, $C_{11}H_{20}$), 1.74–1.89 (br m, 2 H, $CH_2(CH_2N)(CH_2O)$), 2.20–2.60 (overl m's, 6 H, CH_2CO), 3.35–3.43 (br m, 1 H), 3.50–4.15 (overl m's, 14 H), 3.65 (s, 6 H, tris), 4.54 (d, 1 H, J = 8.8, H-1), 5.00–5.09 (br t, 1 H, H-3, J = 10.0); $[\alpha]_D^{20}$ –15.31° (c 0.5, MeOH); MS m/z 973 (MH^+), calcd for $C_{53}H_{100}N_2O_{13}$ 973.73. Anal. of 9·Tris·H_2O ($C_{57}H_{113}N_3O_{17}$) C, H, N.

***N*-[(*R*)-3-(Benzyloxy)tetradecanoyl]-(*S*)-glutamic Acid α-Benzyl Ester (12).** *N*-Ethyldiisopropylamine (2.9 mL, 17.0 mmol) was added to a mixture of (*S*)-glutamic acid α-benzyl ester (2 g, 8.4 mmol), (*R*)-3-(benzyloxy)tetradecanoic acid *N*-hydroxysuccinimide ester (3.62 g, 8.4 mmol) and dimethylformamide (20 mL). The mixture was stirred at room temperature for 17 h. The solvent was removed under high vacuum, and the residue was dissolved in dichloromethane and washed twice with 10% aqueous citric acid and then brine. The organic phase was dried ($MgSO_4$) and the solvent removed. Chromatography of the residue using EtOAc–MeOH–AcOH 98:2:0.25 gave 12 as colorless crystals (2.29 g, 49%): mp 73–74 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3 H, CH_3, J = 6.9), 1.10–1.70 (overl m's, 20 H, $C_{11}H_{20}$), 1.70–1.90 (br m, 1 H, H-β), 2.05–2.34 (overl m's, 3 H, H-β′, CH_2CO), 2.34–2.56 (m, 2 H, CH_2CO), 3.73–3.90 (m, 1 H, HC(OBn)), 4.47–4.60 (ABq, 2 H, Bn, J = 11.3), 4.62–4.73 (m, 1 H, H-α), 5.15 (s, 2 H, CO_2Bn), 6.95 (br d, 1 H, NH, J = 7.5), 7.20–7.40 (m, 10 H, Ph); $[\alpha]_D^{20}$ –7.7° (c 1.3, $CHCl_3$); MS m/z 554 (MH^+), calcd 554.35. Anal. ($C_{33}H_{47}NO_6$) C, H, N.

N-[(R)-3-(Benzyloxy)tetradecanoyl]-(R)-glutamic Acid α-Benzyl Ester (13). Prepared analogous to **12**. The crude product was purified through crystallization from dichloromethane–pentane (82%): mp 69–73 °C; $[\alpha]^{20}_D$ −12.2° (*c* 1.14, $CHCl_3$); MS *m/z* 554 (MH^+), calcd 554.35. Anal. ($C_{33}H_{47}NO_6$) C, H, N.

N-[(R)-3-(Benzyloxy)tetradecanoyl]-(S)-aspartic Acid α-Benzyl Ester (14). Prepared analogous to **12**. The crude product was purified through crystallization from dichloromethane–pentane (86%): mp 66 °C; ^{1}H-NMR ($CDCl_3$) δ 0.89 (t, 3 H, CH_3, *J* = 6.9), 1.20–1.65 (overl m's, 20 H, $C_{11}H_{20}$), 2.47 (d, 2 H, CH_2CO, *J* = 6.3), 2.74 (dd, 1 H, H-β, *J* = 17.5, 5.0), 3.03 (dd, 1 H, H-β′, *J* = 17.5, 5.0), 3.87 (quintet, 1 H, HC(OBn), *J* = 6.3), 4.45–4.55 (ABq, 2 H, Bn, *J* = 12.5), 4.94 (dt, 1 H, H-α, *J* = 8.8, 5.0), 5.12–5.22 (ABq, 2 H, CO_2Bn, *J* = 12.5), 7.23–7.34 (m, 11 H, Ph); $[\alpha]^{20}_D$ +10.5° (c 0.84, $CHCl_3$); MS *m/z* 540 (MH^+), calcd for $C_{32}H_{45}NO_6$ 540.33.

N-[(R)-3-(Benzyloxy)tetradecanoyl]-(R)-aspartic Acid α-Benzyl Ester (15). Prepared analogous to **12**. The crude product was purified through crystallization from dichloromethane–pentane (70%): mp 66–68 °C; $[\alpha]^{20}_D$ −18.4° (*c* 1.10, $CHCl_3$); MS *m/z* 540 (MH^+), calcd for $C_{32}H_{45}N_1O_6$ 540.33.

2-Amino-4,6-O-benzylidene-1-O-[(S)-4-[(benzyloxy)carbonyl]-4-[(R)-3-(benzyloxy)tetradecanamido]butanoyl]-2-N,3-O-bis[(R)-3-(benzyloxy)tetradecanoyl]-2-deoxy-α-D-glucopyranose (16). A solution of DCC (197 mg, 0.96 mmol) in dichloromethane (5 mL) was added in two portions over a period of 1 h to an ice-cooled mixture of 2-amino-4,6-*O*-benzylidene-2-*N*,3-*O*-bis[(*R*)-3-(benzyloxy)tetradecanoyl]-2-deoxy-D-glucose (**10**, 550 mg, 0.61 mmol), **12** (1.05 g, 1.90 mmol), DMAP (5 mg), and CH_2Cl_2 (20 mL). The mixture was stirred for 2 h at 0 °C and allowed to come to room temperature overnight. The precipitated dicyclohexylurea was filtered off, the filtrate was washed successively with aqueous bicarbonate and brine and dried ($MgSO_4$), and the solvent was removed under vacuum. Chromatography (toluene–EtOAc 8:1) of the residue gave **16** (460 mg, 53%) as a white solid: mp 103–106 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 9 H, CH_3, *J* = 6.9), 1.10–1.60 (overl m's, 60 H, $C_{11}H_{20}$), 2.00–2.49 (overl m's, 9 H, CH_2CO, H-β, H-β′), 2.69 (dd, 1 H, CH_2CO, *J* = 15.0, 6.3), 3.70–3.85 (overl m's, 5 H), 3.89–4.00 (m, 1 H, H-5), 4.30–4.65 (overl m's, 8 H), 4.73–4.83 (m, 1 H, H-α), 5.04–5.20 (ABq, 2 H, CO_2Bn, *J* = 12.5), 5.45 (t, 1 H, H-3, *J* = 11.3), 5.49 (s, 1 H, HC(Ph)(O)(O)), 6.17 (d, 1 H, H-1, *J* = 3.8), 6.71 (d, 1 H, NH, *J* = 3.8), 6.75 (d, 1 H, NH, *J* = 3.8), 7.22–7.43 (m, 25 H, Ph); $[\alpha]^{20}_D$ +18.3° (*c* 0.71, $CHCl_3$); MS *m/z* 1435 (MH^+), calcd 1435.93. Anal. ($C_{88}H_{126}N_2O_{14}$) C, H, N.

2-Amino-4,6-O-benzylidene-1-O-[(R)-4-[(benzyloxy)carbonyl]-4-[(R)-3-(benzyloxy)tetradecanamido]butanoyl]-2-N,3-O-bis[(R)-3-(benzyloxy)tetradecanoyl]-2-deoxy-α-D-glucopyranose (17). Prepared analogous to **16** (54%): mp 72–75 °C; $[\alpha]^{20}_D$ +21.8° (*c* 0.59, $CHCl_3$); MS *m/z* 1435.9 (MH^+), calcd 1435.93. Anal. ($C_{88}H_{126}N_2O_{14}$ C, H, N,

2-Amino-4,6-O-benzylidene-1-O-[(S)-3-[(benzyloxy)carbonyl]-3-[(R)-3-(benzyloxy)tetradecanamido]propanoyl]-2-N,3-O-bis[(R)-3-(benzyloxy)tetradecanoyl]-2-deoxy-α-D-glucopyranose (18). Prepared analogous to **16** (52%): mp 112–114 °C; $[\alpha]^{20}_D$ +22.7° (*c* 0.84, $CHCl_3$); MS *m/z* 1421 (MH^+), calcd 1421.91. Anal. ($C_{87}H_{124}N_2O_{14}$) C, H, N.

2-Amino-4,6-O-benzylidene-1-O-[(R)-3-[(benzyloxy)carbonyl]-3-[(R)-3-(benzyloxy)tetradecanamido]propanoyl]-2-N,3-O-bis[(R)-3-(benzyloxy)tetradecanoyl]-2-deoxy-α-D-glucopyranose (19). Prepared analogous to **16** (51%): mp 124–125 °C; $[\alpha]^{20}_D$ +19.9° (*c* 0.56, $CHCl_3$); MS *m/z* 1421 (MH^+), calcd 1421.91. Anal. ($C_{87}H_{124}N_2O_{14}$) C, H, N.

2,3-Diamino-4,6-O-benzylidene-1-O-[(S)-4-[(benzyloxy)carbonyl]-4-[(R)-3-(benzyloxy)tetradecanamido]butanoyl]-2,3-bis-N-[(R)-3-(benzyloxy)tetradecanoyl]-2,3-dideoxy-α-D-glucopyranose (20). Prepared analogous to **16** using **11** (36%): $[\alpha]^{20}_D$ +15.1° (*c* 0.53, $CHCl_3$); MS *m/z* 1434 (MH^+), calcd 1434.95. Anal. ($C_{88}H_{127}N_3O_{13}$) C, H, N.

2,3-Diamino-4,6-O-benzylidene-1-O-4-[(benzyloxy)carbonyl]-4-[(R)-3-(benzyloxy)tetradecanamido]butanoyl]-2,3-bis-N-[(R)-3-(benzyloxy)tetradecanoyl]-2,3-dideoxy-α-D-glucopyranose (21). Prepared analogous to **16** using **11** (63%): ^{1}H-NMR (500 MHz, $CDCl_3$–CD_3OD 1:1, assignments based on Tocsy NMR) δ 0.89 (t, 9 H, CH_3, *J* = 7.0), 1.20–1.65 (br s, 60 H, $C_{11}H_{20}$), 1.83–1.90 (m, 1 H, H-β), 2.16–2.23 (m, 1 H, H-β′), 2.28–2.36 (m, 2 H, CH_2CO), 2.39–2.55 (overl m's, 6 H, CH_2CO), 3.56 (t, 1 H, H-3, *J* = 9.9), 3.71 (t, 1 H, H-6^{ax}, *J* = 10.4), 3.78–3.90 (overl m's, 3 H, H-C(OBn)), 3.93 (ddd, 1 H, H-5, *J* = 9.8, 9.8, 4.8), 4.23 (dd, 1 H, H-6^{eq}, *J* = 4.9, 10.0), 4.31 (dd, 1 H, H-2, *J* = 3.5, 11.4), 4.35–4.41 (ABq, 2 H, OBn, *J* = 11.8), 4.44–4.51 (ABq, 2H, OBn, *J* = 11.5), 4.47 (t, 1 H, H-4, *J* = 10.5), 4.51 (br s, 2 H, OBn, 4.58 (dd, 1 H, H-α, *J* = 4.8, 9.3), 5.10–5.17 (ABq, 2 H, OBn, *J* = 12.1), 5.40 (s, 1 H, HC(Ph)(O)(O)), 6.16 (d, 1H, H-1, *J* = 3.5), 7.17–7.39 (m, 25 H, Ph); $[\alpha]^{20}_D$ +7.1° (*c* 1.2, $CHCl_3$); MS *m/z* 1434 (MH^+), calcd 1434.95. Anal. ($C_{88}H_{127}N_3O_{13}$) C, H, N.

General Procedure for the Deprotection of 16–21 To Give 22–27, Respectively. A mixture of 0.3 mmol of the compound (**16–21**), tetrahydrofuran (90 mL), water (10 mL), and 10% Pd/C (150 mg) was stirred under hydrogen atmosphere until completion of the reaction (1 d). The catalyst was filtered off on a bed of Celite, and the filtrate was filtered through a membrane filter (0.02-μm pore size) and the volatiles stripped off under vacuum. The residue was suspended in water and lyophilized. The lyophilizate was washed with ether to leave behind the pure product (**22–27**, respectively), yields 97–99%. The Tris salts were prepared through sonicating a mixture of the compound (0.3 mmol), tris (0.3 mmol) and water (75 mL) for 5 min, followed by lyophilization.

2-Amino-1-O-[(S)-4-carboxy-4-[(R)-3-hydroxytetradecanamido]butanoyl]-2-N,3-O-bis[(R)-3-hydroxytetradecanoyl]-2-deoxy-α-D-glucopyranose (22) tris salt: $[\alpha]^{20}_D$ +8.5° (*c* 1.03, $CHCl_3$–MeOH 1:1); MS *m/z* 987.5 (MH^+), calcd for $C_{53}H_{99}N_2O_{14}$ 987.70. Anal. of **22**·Tris·$3H_2O$ ($C_{57}H_{115}N_3O_{20}$) C, H, N.

2-Amino-1-O-[(R)-4-carboxy-4-[(R)-3-hydroxytetradecanamido]butanoyl]-2-N,3-O-bis[(R)-3-hydroxytetradecanoyl]-2-deoxy-α-D-glucopyranose (23): mp 154–155 °C (dichloromethane–ethanol); ^{1}H-NMR (500 MHz, $CDCl_3$–CD_3OD 1:1) δ 0.89 (t, 9 H, CH_3, *J* = 6.9), 1.26–1.55 (br m, 60 H, $C_{11}H_{20}$), 1.88–1.95 (m, 1 H, H-β), 2.27–2.39 (overl m's, 9 H, CH_2CO, H-β′), 3.70–3.83 (overl m's, 4 H, H-4,5,6,6′), 3.86–3.91 (m, 1 H, HC(OH)(CH_2CO)), 3.96–4.03 (m, 1 H, HC(OH)(CH_2CO)), 4.35 (dd, 1 H, H-2, *J* = 11.0, 3.5), 4.57 (dd, 1 H, H-α, *J* = 4.7, 9.6), 5.19 (dd, 1 H, H-3, *J* = 8.6, 11.0), 6.19 (d, 1 H, H-1, *J* = 3.5); $[\alpha]^{20}_D$ +24.9° (*c* 1.17, $CHCl_3$–MeOH 1:1); MS *m/z* 987 (MH^+), calcd 987.70. Anal. ($C_{53}H_{98}N_2O_{14}$) C, H, N.

2-Amino-1-O-[(S)-3-carboxy-3-[(R)-3-hydroxytetradecanamido]propanoyl]-2-N,3-O-bis[(R)-3-hydroxytetradecanoyl]-2-deoxy-α-D-glucopyranose (24) tris salt: $[\alpha]^{20}_D$ +37.6° (*c* 0.55, $CHCl_3$–MeOH 1:1); MS *m/z* 973 (MH^+), calcd for $C_{52}H_{97}N_2O_{14}$ 973.69. Anal. of **24**·tris·$2H_2O$ ($C_{56}H_{111}N_3O_{18}$) C, H, N.

2-Amino-1-O-[(R)-3-carboxy-3-[(R)-3-hydroxytetradecanamido]propanoyl]-2-N,3-O-bis[(R)-3-hydroxytetradecanoyl]-2-deoxy-α-D-glucopyranose (25) tris salt: $[\alpha]^{20}_D$ 0° (*c* 0.5, $CHCl_3$–MeOH 1:1); MS *m/z* 973 (MH^+), calcd for $C_{52}H_{97}N_2O_{14}$ 973.69. Anal. of **25**·tris·$2H_2O$ ($C_{56}H_{111}N_3O_{18}$) C, H, N.

2,3-Diamino-1-O-[(S)-4-carboxy-4-[(R)-3-hydroxytetradecanamido]butanoyl]-2,3-bis-N-[(R)-3-hydroxytetradecanoyl]-2,3-dideoxy-α-D-glucopyranose (26): $[\alpha]^{20}_D$ +21.1° (*c* 0.64, $CHCl_3$–MeOH 1:1); MS *m/z* 986.5 (MH^+), calcd 986.73. Anal. ($C_{53}H_{99}N_3O_{13}$) C, H, N.

2,3-Diamino-1-O-[(R)-4-carboxy-4-[(R)-3-hydroxytetradecanamido]butanoyl]-2,3-bis-N-[(R)-3-hydroxytetradecanoyl]-2,3-dideoxy-α-D-glucopyranose (27): $[\alpha]^{20}_D$ +14.2° (*c* 0.55, $CHCl_3$–MeOH 1:1); MS *m/z* 986 (MH^+), calcd 986.73. Anal. ($C_{53}H_{99}N_3O_{13}$) C, H, N.

Biology. Enhancement of Nonspecific Resistance (NSR). 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 iv with the test compounds (12.5 mg/kg), and one group was treated iv with LPS (*Salmonella abortus equi*, Sigma Chemical Co., 250 μg/kg) to be used as control. Finally, the mice were infected on day 0 by intravenous inoculation with *Pseudomonas aeruginosa* (Δ12), inoculum = 4×10^5 cfu (LD_{95}). The table shows the survival rate after an observation period of 4 days. The buffer-treated and infected control mice died within 48 h after infection. For compounds inducing a high survival rate, the ED_{50} was determined and calculated using Probit[35] analysis.

Endotoxin (ET) Shock Induction. Lethal toxicity in galactosamine-sensitized mice was tested by the method of Galanos.[31] Serial dilutions of test compounds (either in pyrogen-free saline or in isotonic glucose solution containing 1% ethanol) 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[35] analysis.

Induction of TNF-α. Induction of TNF-α and cultivation of mouse bone marrow-derived macrophages. Bone marrow cells were flushed from femurs of $B_6D_2F_1$ mice and cultured at 37 °C in an atmosphere of 5% CO_2 for 8-10 days in Teflon bags. Dulbecco's modified Eagle's medium (DMEM) H 21 supplemented with 10% fetal calf serum, 5% horse serum, antibiotics, and 20% L929 cell culture supernatant as the source of CSF-1 were used.[36] The differentiated macrophages were washed twice and resuspended in RPMI 1640 medium supplemented with 50 units/mL penicillin, 50 μg/mL streptomycin, 2 mM glutamine, and 5% fetal calf serum. The cell suspension was adjusted to 10^6 cells/mL, and aliquots of 1 mL/well were incubated on Costar plates for 3 h at 37 °C; the nonadherent cells were washed off, and the adherent cells were incubated overnight in the presence or absence of 100 units/mL interferon γ. For the induction of TNF-α, the cells were washed twice in prewarmed serum-free medium and incubated for 4 h with the appropriate test samples dissolved in 1-mL amounts of serum-free medium. The supernatants were harvested and kept at -20 °C. TNF-α levels in the supernatants were determined by the cytotoxicity of TNF for actinomycin-D-treated L929 cells as described elsewhere.[32,37]

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Registry No. 1, 142981-91-7; 2, 142981-92-8; 3, 142981-93-9; 4, 142981-94-0; 5, 142981-95-1; 6, 142981-96-2; 7, 142981-97-3; 8, 142981-98-4; 8 Tris salt, 142982-12-5; 9, 142981-99-5; 9 Tris salt, 142982-13-6; 10, 142982-00-1; 11, 141692-86-6; 12, 142982-01-2; 13, 142982-02-3; 14, 142982-03-4; 15, 142982-04-5; 16, 142982-05-6; 17, 143060-80-4; 18, 142982-06-7; 19, 143060-81-5; 20, 142982-07-8; 21, 143060-82-6; 22, 142982-08-9; 22 Tris alt, 143060-86-0; 23, 143060-83-7; 23 Tris salt, 143119-65-7; 24, 142982-09-0; 24 Tris salt, 143062-03-7; 25, 143060-84-8; 25 Tris salt, 143119-71-5; 26, 142982-10-3; 26 Tris salt, 143060-87-1; 27, 143060-85-9; 27 Tris salt, 143119-66-8; benzyl 2-[(3-hydroxypropyl)amino]acetate, 142982-11-4; (R)-3-(benzyloxy)tetradecanoic acid N-hydroxysuccinimide ester, 101649-06-3; (R)-3-(benzyloxy)tetradecanoic acid, 87357-67-3.

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Synthesis and Antibacterial Activity of 1-(Substituted pyrrolyl)-7-(substituted amino)-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acids

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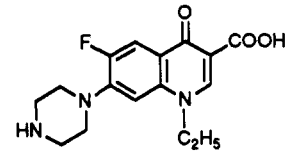
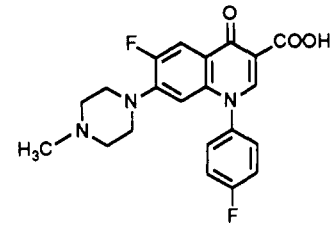
Seventeen quinolone compounds characterized by having a fluorine atom at the 6-position, a substituted amino at the 7-position, and a substituted pyrrolyl at the 1-position were synthesized for the first time. The in vitro antibacterial activities of these compounds against *Escherichia coli* and *Staphylococcus aureus* were tested. Among these agents obtained, compound **24** showed significantly enhanced activity against *S. aureus*. The results indicate that there is much room for modifications at the N-1 position.

Quinolone antibacterial agents are among the most attractive drugs in the anti-infective chemotherapy field. Although their structure-activity relationship (SAR) has been extensively studied,[1-7] the SAR for N-1 has not reached a clear conclusion. Studies by Koga indicate that the antibacterial activity of quinolones is greatly influenced by the steric bulk of the N-1 substituent. The best sterimol length is 4.17 Å with any groups bigger or smaller than that value decreasing the activity.[8] Such is the case that norfloxacin containing ethyl whose *L* is 4.14 Å at the N-1 position has good activity. Later, Chu et al. discovered that quinolones with fluorophenyl at the N-1 position also possess excellent activity.[9,10] This substitution pattern was not considered in Koga's early study[8] because the size of the benzene ring would have likely made it inactive. It seems that the electronic properties of 1-substituents, in addition to their steric bulk, also play an important role in the potency.[11] Therefore, when an aromatic five-membered heterocycle was introduced at the N-1 position,



norfloxacin



difloxacin

the activity of the quinolones is definitely different from that with a phenyl substituent. To date, modifications

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