to ca. 175 °C under argon for 22 h. After cooling to room temperature, the reaction mixture was dissolved in methylene chloride (1 L) and washed with 10% aqueous $KHCO_3$ (2 × 1 L) and water (2 × 1 L). The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed on silica gel (500 g) with methanol–methylene chloride (1:9) as eluent and afforded 19 g (17%) of **20**. Recrystallization from ethanol gave **20** as an off-white solid: mp 235–237 °C; IR (Nujol) 3100, 1600, 1545, 1510, and 1310 cm^{-1}; NMR (300 MHz, DMSO-d_6) δ 2.32 (s, 3), 2.57 (q, 2), 3.02 (s, 3), 3.94 (t, 2), 5.84 (t, 1), 6.70 (s, 1), 7.24 (d, 2), 7.44 (d, 2), 9.90 (s, 1). Anal. ($C_{15}H_{17}N_3O_2S$) C, H, N.

3-Methyl-8-[4-[(methylsulfonyl)amino]phenyl]-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine (21). A 500-mL Parr bottle was charged with **20** (5.0 g, 16.5 mmol), 2.0 g of 10% palladium on activated carbon, and 100 mL of 1 N NaOH. The mixture was hydrogenated at ca. 50 psi for 16 h at room temperature. The catalyst was collected by filtration, neutralized by using solid NH_4Cl, and extracted with methylene chloride (2 × 100 mL). Drying the organic extracts (Na_2SO_4) and concentrating at reduced pressure gave 5.0 g of **21** as a gummy solid. Recrystallization from ethyl acetate gave 4.6 g (92%) of **21** as a white solid: mp 206–207 °C; IR (Nujol) 1505, 1460, 1340, and 1155 cm^{-1}; NMR (300 MHz, DMSO-d_6) 1.80–2.05 (m, 4), 2.34 (s, 3), 2.97 (s, 3), 3.75 (m, 1), 3.96 (m, 2), 6.09 (s, 1), 7.15 (d, 2), 7.17 (d, 2), 9.68 (s, 1). Anal. ($C_{15}H_{19}N_3O_2S$) C, H, N.

5,6-Dihydro-2,3-dimethyl-8-[4-[(methylsulfonyl)amino]phenyl]imidazo[1,5-*a*]pyridinium Iodide (22). A mixture of **20** (6.0 g, 19.8 mmol) and methyl iodide (13.0 mL, 208.8 mmol) in 50 mL of methanol was heated in a pressure bottle for 20 h at ca. 60 °C. After cooling to room temperature, the solution was concentrated at reduced pressure and the resulting solid (6.2 g) slurried in ethanol. Recrystallization from ethanol afforded 4.6 g (52%) of **22** as a white solid: mp 237–238 °C; IR (Nujol) 2940, 1605, 1595, 1505, 1340, and 1170 cm^{-1}; NMR (300 MHz, DMSO-d_6) δ 2.64 (s, 3), 2.72 (q, 2), 3.04 (s, 3), 3.75 (s, 3), 4.20 (t, 2), 6.30 (t, 1), 7.28 (d, 2), 7.45 (d, 2), 7.61 (s, 1), 9.95 (s, 1). Anal. [($C_{16}H_{20}N_3O_2S$)$^+I^-$] C, H, N.

2,3-Dimethyl-8-[4-[(methylsulfonyl)amino]phenyl]-5,6,7,8-tetrahydroimidazo[1,5-a]pyridinium Iodide (23). A mixture of **21** (6.0 g, 19.6 mmol) and methyl iodide (15.0 mL, 240.9 mmol) in 100 mL of methanol was heated in a pressure bottle for 20 h at ca. 60 °C. After cooling to room temperature, the solution was concentrated at reduced pressure and the resulting solid (9.2 g) slurried in ethanol. Recrystallization from ethanol afforded 6.5 g (74%) of **23** as a white solid: mp 187–188 °C; IR (Nujol) 3090, 1510, 1330, and 1155 cm^{-1}; NMR (300 MHz, DMSO-d_6) δ 1.80–2.10 (m, 4), 2.56 (s, 3), 3.00 (s, 3), 3.69 (s, 3), 3.99–4.30 (m, 3), 7.04 (s, 1), 7.20 (d, 2), 7.25 (d, 2), 9.76 (s, 1). Anal. [($C_{16}H_{22}N_3O_2S$)$^+I^-$] C, H, N.

Pharmacology. Intracellular and extracellular elecrophysiological profiles, intradueodenal bioavailability, antiarrhythmic efficacy (PES model) and β-adrenergic receptor binding studies were performed according to previously established procedures.[3] Samples of sotalol and clofilium were prepared by in-house synthesis.

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Registry No. **2a**, 64488-52-4; **2b**, 5317-90-8; **3a**, 110698-54-9; **3c**, 110698-73-2; **4c** (isomer 1), 110698-74-3; **4c** (isomer 2), 110698-75-4; **5**, 41104-10-3; **6**, 110698-55-0; **7**, 110698-56-1; **8**, 110698-57-2; **8**·HCl, 110698-58-3; **9**, 110698-59-4; **10**, 30148-20-0; **11**, 110698-60-7; **12**, 110698-61-8; **13**, 5317-89-5; **14**, 110698-62-9; **15**, 110698-63-0; **16**, 110698-64-1; **17**, 110698-76-5; **18**, 1197-22-4; **19**, 108060-61-3; **20**, 108060-52-2; **21**, 108060-55-5; **22**, 110698-65-2; **23**, 110698-66-3; thiazole, 288-47-1; 1-methylimidazole, 616-47-7; 1-[2-[4-(acetyloxy)phenyl]-2-oxoethyl]-3-methyl-1*H*-imidazolium bromide, 110698-67-4; 2-methyl-2-imidazoline, 534-26-9; *N*-[4-[(1-methyl-1*H*-imidazol-2-yl)carbonyl]phenyl]methanesulfonamide, 110698-68-5; *N*-[4-[hydroxy(1-methyl-1*H*-imidazol-2-yl)-methyl]phenyl]methanesulfonamide, 110698-69-6; *N*-[4-(1-oxoethyl)phenyl]-*N*-(phenylmethyl)methanesulfonamide, 110698-70-9; *N*-[4-[2-(1*H*-imidazol-1-yl)-1-oxoethyl]phenyl]-*N*-(phenylmethyl)methanesulfonamide, 110698-71-0; imidazole, 288-32-4; 4-chlorobutyryl chloride, 4635-59-0; 2-methylimidazole, 693-98-1; *N*-[4-(2,2-dibromo-1-oxoethyl)phenyl]-*N*-(phenylmethyl)-methanesulfonamide, 110698-72-1; *N*-[4-[1-hydroxy-2-(4,5-dihydro-2-methyl-1*H*-imidazol-1-yl)ethyl]phenyl]methanesulfonamide, 111323-42-3.

Design and Synthesis of Peptide Derivatives of a 3-Deoxy-D-*manno*-2-octulosonic Acid (KDO) Analogue as Novel Antibacterial Agents Acting upon Lipopolysaccharide Biosynthesis

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On the basis of the knowledge that the amino acid **3** (8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonic acid) is a potent inhibitor of 3-deoxy-*manno*-octulosonate cytidylyltransferase, attempts were made to design derivatives that would act as antibacterials against Gram-negative bacteria by inhibiting lipopolysaccharide biosynthesis. Compound **3** and the derivatives **15** and **16** containing an additional amino acid were not lethal to bacteria. However, compounds **17–22**, which contain a N-terminally linked dipeptide, exhibited good antibacterial activity in vitro on testing against strains of the Gram-negative bacteria *Escherichia coli* and *Salmonella typhimurium*. They have no activity against Gram-positive bacteria such as *Staphylococcus aureus*.

Since the outer membrane of Gram-negative bacteria is important both to pathogenesis and resistance to existing antimicrobial agents,[1,2] the rational design of inhibitors of its biosynthesis should produce novel agents effective only against Gram-negative bacteria.[3] The outer leaflet of this membrane contains lipopolysaccharide[4] (LPS), which provides an attractive target, since it contains components unique to Gram-negative bacteria. Among these is the sugar 3-deoxy-D-*manno*-2-octulosonic acid[5] (KDO, **1**),

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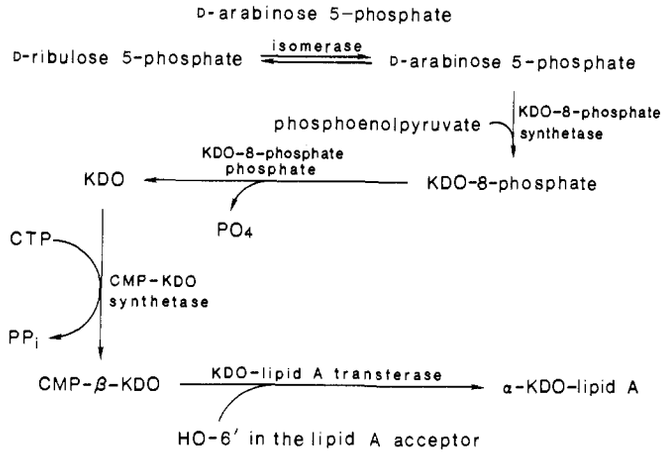
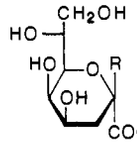
0022-2623/87/1830-2309$01.50/0

Scheme I

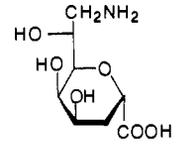


which links the hydrophilic carbohydrate polymer region, the core plus the O-side chain, to the hydrophobic lipid A moiety, a phosphorylated and acylated glucosamine disaccharide. Lipid A is embedded in the outer membrane, whereas the carbohydrate chain extends outward into the environment.

Mutants defective in KDO biosynthesis are not viable,[6] and since the biosynthesis and incorporation of KDO (Scheme I has been well studied,[5,7] we chose the KDO enzymes[8,9] as prime targets for the design of novel antibacterials.[10a] The enzyme 3-deoxy-*manno*-octulosonate cytidylyltransferase[9] (CMP-KDO synthetase; EC 2.7.7.38) converts KDO to the nucleotide sugar cytidine 5′-monophosphate KDO (CMP-KDO). This is thought to be the rate-limiting enzyme in KDO incorporation[7,8] and is therefore an attractive target. The primary structure of the enzyme from *Escherichia coli* was recently reported[10b] and NMR[11] as well as inhibition[12a] studies have shown the β-pyranose form of KDO (**1**) to be the enzyme substrate.



1, R = OH(β-KDO)
2, R = H



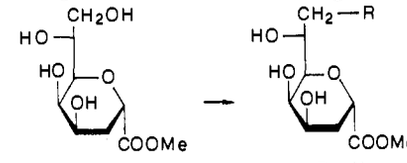
3

Bigham et al., exploiting a similar approach, elected to work with inhibitors of arabinose 5-phosphate isomerase.[13] None of the inhibitors reported displayed antibacterial activity. Most of the other enzymes involved in the KDO pathway have been examined as targets for potential inhibitors, but no such compounds have been reported.[8]

CMP-KDO synthetase was purified from *Salmonella typhimurium* SL 1102 and used to test structural analogues of KDO for inhibitory activity.[14,15] The analogue 2-deoxy-KDO (**2**) was found to be a potent inhibitor of CMP-KDO synthetase,[12] but failed to kill bacteria. The lack of antibacterial activity was suspected to result from an inability of the inhibitor to cross the cytoplasmic membrane. In an attempt to circumvent this barrier, the 8-amino analogue **3** was synthesized since such a compound would be suitable for linkage to amino acids or small peptides, thereby exploiting ubiquitous bacterial peptide permeases[16] to smuggle[17] the inhibitor into the cytoplasm of the bacterium. The amino acid **3** was shown to possess equivalent inhibitory activity to **2**.[10a,18] This permitted the synthesis of a number of transport forms able to deliver inhibitor to the cell interior of the bacterium. The results are reported here.

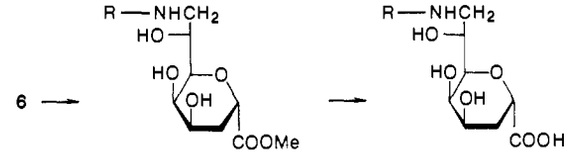
Results

Chemistry. The ester **4** is available in six steps starting from KDO[14] or in five steps starting from D-mannose diacetonide.[19] It was readily converted into the azide **5** by the Ph_3P–CBr_4–LiN_3 method[20] and this, in turn, was hydrogenated to give the amino ester **6** in good overall yield.



4 **5**, R = N_3 **6**, R = NH_2

Coupling of **6** with various Cbz-protected L,L-dipeptides or L-amino acids afforded the protected peptides **7**–**14**. Deprotection was performed in two steps by first hydrolyzing the methyl ester (LiOH) and then converting the lithium-carboxylate into the ammonium salt, which was hydrogenolyzed over Pd–C.



7, R = Cbz-Ala	**15**, R = Ala
8, R = Cbz-Met	**16**, R = Met
9, R = Cbz-Ala-Ala	**17**, R = Ala-Ala
10, R = Cbz-Val-Met	**18**, R = Val-Met
11, R = Cbz-Pro-Met	**19**, R = Pro-Met
12, R = Cbz-Arg-Nva	**20**, R = Arg-Nva
13, R = Cbz-Ala-Phe	**21**, R = Ala-Phe
14, R = Cbz-Lys-Leu	**22**, R = Lys-Leu

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Table I. In Vitro Antibacterial Activities

Compound	minimum inhibitory concentration, μM		
	S. typhimurium SL 1102	*S. typhimurium* LT2 168	*E. coli* ATCC 11303
15	>2000	>2000	>2000
16	>2000	>2000	>2000
17	4	60	4
18	16	60	4
19	16	120	30
20	2	4	1
21	250	60	8
22	30	30	8
ampicillin	2	0.3	1
gentamicin	<0.05	<0.05	<0.05

The peptide derivatives **15–22** were obtained as amorphous solids, which were identified by ^1H and ^{13}C NMR and by high-resolution FAB-MS. Purity was ascertained by TLC and, in part, by ^{13}C NMR.

Biology. The peptides were tested in vitro for minimum inhibitory concentration (MIC) values against the bacterial species shown in Table I. The bacteria were grown in a medium deficient in peptides since the latter have been shown to interfere with the uptake of peptide antimicrobials.[21] In similar determinations using a medium containing peptides, bacterial inhibition could not be observed. As expected, Gram-positive bacteria, e.g. *Staphylococcus aureus*, were unaffected by all the test compounds. The first synthesized compound in the present series, namely the dialanine derivative **17**, was also tested for activity against certain other strains and species in peptide-free defined medium. The MIC values observed for *E. coli* J5, *E. coli* Δ120, *S. typhimurium* AG701i50, *Klebsiella aerogenes* NCTC 1128, *Pseudomonas aeruginosa*, and *Proteus mirabilis* 4 were 65, 1000, 4, 125, >2000, and >2000 μM, respectively.

Discussion

The observation[18] that the amino acid **3** has equivalent inhibition to the hydroxy acid **2** suggested that the linkage of peptides to **3** could produce a compound resembling a natural substrate for the bacterial peptide permeases.[16] The attachment of L-amino acids to normally impermeant toxic molecules has been used successfully in the past.[17,21] Subsequent intracellular hydrolysis then releases the active principle, which often has been a toxic amino acid, such as, for example, in the synthetic dipeptide alafosfalin,[21] in peptides containing 2-aminopimelic acid,[22] and in certain antibiotics.[17b] Other transport devices, in which the molecule to be transported is not peptide-linked, have also been described.[23]

The addition of a single N-terminal amino acid as in **15** and **16** did not affect the bacterial growth. Addition of a dipeptide gave rise to compounds **17–22**, which possessed antibacterial activity and killed the bacteria by inhibiting KDO incorporation and hence LPS biosynthesis. This is the first report of such compounds. Detailed studies[24] using compound **17** have revealed that the compound is taken up into *E. coli* via the oligopeptide permease (opp) and that cytoplasmic aminopeptidases slowly liberate **3**. This hydrolysis is two-thirds complete within 30 min. The death of the bacterium has been shown to be the direct result of a selective inhibition of LPS biosynthesis.[24]

The in vitro antibacterial activities (MIC) of the present peptide derivatives are surprisingly good against the *Salmonella* and *E. coli* strains tested. Some of them even approach the antibacterial activity of ampicillin (Table I).

The above data does not allow any extensive discussion about structure–activity relationships. However, it is interesting to note the dramatic increase in activity, which is roughly correlated to transport rate,[24] in going from derivatives containing a single amino acid to compounds having a N-terminal dipeptide chain. The dipeptide arginylnorvalyl confers the most favorable transport–cleavage properties on its conjugate, i.e., compound **20** (Table I).

The compounds presented here exhibit good antibacterial activity in vitro, but are less useful in vivo since they are readily degraded by mammalian peptidases (unpublished results). However, they represent a series of compounds acting via a novel inhibition mechanism with potential for development as a new class of chemotherapeutic agents.

Experimental Section

Melting points were determined in open capillary tubes and are uncorrected. Optical rotations were measured at 20 °C with an Optical Activity AA 100 polarimeter. IR spectra were recorded with a Perkin-Elmer 298 spectrometer. ^{13}C NMR spectra were recorded with a JEOL FX200 instrument using tetramethylsilane (TMS) as internal standard in $CDCl_3$, CD_3OD, and DMF-d_7 and *tert*-butyl alcohol (δ = 30.6) in D_2O. High-resolution fast-atom-bombardment (FAB) mass spectra were obtained on a JEOL DX 303 mass spectrometer. Thin-layer chromatography (TLC) was performed on precoated plates of Merck silica gel 60 F_{254} with visualization using ninhydrin or by charring with sulfuric acid. Column chromatography was performed on Merck silica gel 60 (0.040–0.063 mm). All solvents used were of anhydrous quality and kept over 3-Å or 4-Å molecular sieves. DMF was distilled from P_2O_5 and then kept over 4-Å molecular sieves. Solutions were evaporated in vacuo at a temperature not exceeding 30 °C. Elemental analyses were carried out at the Department of Analytical Chemistry, University of Lund.

The following compounds were purchased from BACHEM Feinchemikalien AG (Cbz = benzyloxycarbonyl): *N*-Cbz-L-methionine, *N*-Cbz-L-norvaline, *N*-Cbz-L-alanyl-L-alanine, *N*-Cbz-L-alanyl-L-phenylalanine, N^{α},N^{ϵ}-di-Cbz-L-lysyl-L-leucine, *N*-Cbz-L-prolyl-L-methionine, *N*-Cbz-L-valyl-L-methionine, *N*-Cbz-L-alanine succinimido ester, and N^{α},N^{G},N^{G}-tri-Cbz-L-arginine succinimido ester.

General Procedure A. Hydrolysis of the Methyl Esters. Hydrolysis was performed with lithium hydroxide (3–4 equiv) in the solvent mixtures indicated until completion according to TLC (0.5–2 h). The mixture was then passed through an ammonium-saturated ion-exchange resin (DOWEX 50 W × 8).

General Procedure B. Hydrogenolysis of the Benzyloxycarbonyl Group. All the catalytic hydrogenations were performed with prehydrogenated 10% palladium on charcoal as a catalyst (0.1–0.5 weight equiv) in a Parr apparatus at 3–4 bar for 1–3 h in the solvents indicated. The catalyst was filtered off and carefully washed. The filtrate was concentrated to dryness.

General Procedure C. Preparation of Activated Succinimido Esters. A solution of the *N*-benzyloxycarbonyl-protected compound and *N*-hydroxysuccinimide (1 equiv) in THF was stirred at 0 °C. Dicyclohexylcarbodiimide (1 equiv) dissolved in THF was added, and after stirring at 0–5 °C for 1–3 h, the mixture was filtered and the filtrate was evaporated in vacuo.

Methyl 2,6-Anhydro-8-azido-3,8-dideoxy-D-*glycero*-D-*talo*-octonate (5). Methyl 2,6-anhydro-3-deoxy-D-*glycerol*-D-*talo*-octonate[14] (**4**) (20.0 g, 0.085 mol) and lithium azide (27.4 g, 0.56 mol) were dissolved in DMF (460 mL) under a nitrogen

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atmosphere by slightly warming the mixture. Tetrabromomethane (53.8 g, 0.16 mol) was added, and when all had dissolved, the mixture was cooled to 0 °C and triphenylphosphine (42.4 g, 0.16 mol) was added in several portions. The solution was stirred at room temperature overnight. After addition of methanol (10 mL) and concentration of the mixture to dryness, the residue was triturated with diethyl ether (4 × 700 mL) to remove part of the triphenylphosphine oxide formed. Chromatography on silica gel of the solid residue with EtOAc/Tol/MeOH (7:3:0.5) followed by crystallization from EtOAc/MeOH gave pure **5** (18.3 g, 82%): mp 141.5–142.0 °C; R_f 0.41 ($CHCl_3$/MeOH, 8:1); $[\alpha]^{20}_D$ + 45° (*c* 1.0, MeOH); IR 2100 (azide), 1740 cm^{-1} (ester); ^{13}C NMR (CD_3OD) δ 173.6 (C-1), 76.5, 73.8, 69.8, 67.8, 67.7 (C-2,4,5,6,7) 55.6 (C-8), 52.7 (OCH_3), 29.4 (C-3). Anal. ($C_9H_{15}N_3O_6$) C, H, N.

Methyl 8-Amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonate (6). Compound **5** (4.0 g, 15 mmol) dissolved in MeOH/THF/EtOAc (1:2.5:8) (115 mL) was hydrogenated according to general procedure B. The catalyst was washed with methanol and concentration of the filtrate to dryness gave **6** (3.2 g, 90%) as a white powder: R_f 0.35 (MeOH/triethylamine, 9:1); ^{13}C NMR (CD_3OD) δ 173.8 (C-1), 76.5, 73.7, 70.2, 68.1, 68.0, 68.0 (C-2,4,5,6,7), 52.6 (OCH_3), 45.7 (C-8), 29.6 (C-3); FAB-MS, [M + H]$^+$ at *m/z* 236.1182 (calcd 236.1134).

Methyl *N*-[*N*-(Benzyloxycarbonyl)-L-alanyl]-8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonate (7). *N*-Cbz-L-alanine succinimido ester (0.20 g, 0.62 mmol) and **6** (0.19 g, 0.82 mmol) were dissolved in MeOH/THF (1:1) (4 mL), and the mixture was stirred at room temperature for 3 h. Concentration and purification on silica gel with $CHCl_3$/MeOH (10:1) gave **7** (0.15 g, 54%): R_f 0.25 ($CHCl_3$/MeOH, 8:1); ^{13}C NMR (CD_3OD) δ 175.5, 173.6, 157.5 (C=O), 137.4, 129.1, 128.7, 128.5 (aromatic), 76.7, 73.3, 68.7, 67.4, 67.4 (C-2,4,5,6,7), 67.4 (CH_2Ph), 52.6, 51.8 (CH, OCH_3), 43.7 (C-8), 29.2 (C-3), 18.5 (CH_3).

***N*-L-Alanyl-8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonic Acid (15).** Compound **7** (0.13 g, 0.3 mmol) was hydrolyzed according to general procedure A in THF/H_2O (1:1) (4 mL) followed by ion exchange in THF/H_2O (1:3). Hydrogenation according to general procedure B was performed in water. The product was purified by ion-exchange chromatography (H^+) with water as eluent and then with 2 M ammonium hydroxide. Concentration of appropriate fractions gave **15** as an amorphous solid (47 mg, 53%): R_f 0.39 (2-propanol/H_2O (3:1), single spot with ninhydrin and sulfuric acid); $[\alpha]^{20}_D$ +85.5° (*c* 1.5, H_2O); ^{13}C NMR (D_2O) δ 179.2, 172.0 (C=O), 75.4, 75.2, 68.0, 67.7, 67.0 (C-2,4,5,6,7), 50.2 (CH), 43.5 (C-8), 29.4 (C-3), 17.6 (CH_3); FAB-MS, [M + H]$^+$ at *m/z* 293.1372 (calcd 293.1349).

Methyl *N*-[*N*-(Benzyloxycarbonyl)-L-methionyl]-8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonate (8). *N*-Cbz-L-methionine succinimido ester[25] (1.0 g, 2.9 mmol) prepared according to general procedure C and **6** (0.6 g, 2.6 mmol) were dissolved in MeOH/THF (1:1) (12 mL), and the mixture was stirred at room temperature for 1 h. Concentration and purification on silica gel with $CHCl_3$/Tol/MeOH (5:2:0.75) followed by precipitation from a $CHCl_3$/MeOH solution with water gave **8** (0.24 g, 75%): mp 160–163 °C; R_f 0.3 ($CHCl_3$/Tol/MeOH, 5:1:2); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 173.7, 173.2, 157.0 (C=O), 136.6, 128.7, 128.4, 128.1 (aromatic), 75.8, 72.7, 68.3, 67.3, 66.9, 66.7, (C-2,4,5,6,7, CH_2Ph), 54.9 (CH), 52.6 (OCH_3), 43.3 (C-8), 32.5, 30.4, 28.8 (C-3, 2 CH_2-Met), 15.3 (SCH_3).

***N*-L-Methionyl-8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonic Acid (16).** Compound **8** (0.24 g, 0.48 mmol) was hydrolyzed in THF/H_2O (1:1) (14 mL) according to general procedure A (ion exchange was performed in water) and hydrogenation was performed in water (5 mL) according to general procedure B. The product was purified by ion-exchange chromatography (H^+) first with water as eluent and then with 2 M ammonium hydroxide. Concentration of appropriate fractions gave **16** as an amorphous solid (60 mg, 35%): R_f 0.63 (2-propanol/H_2O (3:1), single spot with ninhydrin and sulfuric acid); $[\alpha]^{20}_D$ + 71.1° (*c* 2.0, H_2O); ^{13}C NMR (D_2O) δ 179.4, 170.7 (C=O), 75.5, 75.2, 67.9, 67.7, 67.1 (C-2,4,5,6,7), 53.8 (CH), 43.7 (C-8, 31.0, 29.4, 29.1 (C-3, 2 CH_2-Met), 15.0 (SCH_3); FAB-MS, [M + H]$^+$ at *m/z* 353.1377 (calcd 353.1382).

Methyl *N*-[*N*-(Benzyloxycarbonyl)-L-alanyl-L-alanyl]-8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonate (9). *N*-Cbz-L-alanyl-L-alanine succinimido ester[26] (2.3 g, 5.8 mmol), prepared according to general procedure C and purified by crystallization from 2-propanol, was dissolved in THF/MeOH (3:2) (10 mL) and added to a solution of **6** (1.5 g, 6.0 mmol) in THF/MeOH (2:5) (14 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and at room temperature for 2 h. The separated product was filtered off and the filtrate was concentrated. The residue was triturated with water, filtered, and dried over P_2O_5. This gave **9** (2.25 g, 75%): mp 210–215 °C; R_f 0.47 ($CHCl_3$/MeOH, 4:1); ^{13}C NMR (DMF-d_7) δ 173.3, 173.1, 156.9 (C=O), 138.9, 138.0, 129.0, 128.4, 128.3 (aromatic), 77.2, 73.1, 68.9, 67.3, 67.3 (C-2,4,5,6,7), 66.4 (CH_2Ph), 52.2, 51.5, 49.5 (OCH_3, 2 CH) 44.1 (C-8), 29.6 (C-3), 18.5 (CH_3).

***N*-(L-Alanyl-L-alanyl)-8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonic Acid (17).** Compound **9** (2.25 g, 4.4 mmol) was hydrolyzed in THF/H_2O (1:1) (2.6 mL) according to general procedure A (ion exchange with water as eluent) and hydrogenation was performed in water (15 mL) according to general procedure B. Crystallization from warm methanol gave **17** (1.1 g, 69%): mp 190 °C dec; R_f 0.33 (2-propanol/H_2O, 3:1, single spot with ninhydrin and sulfuric acid); $[\alpha]^{20}_D$ + 70.3° (*c* 1.6, H_2O); ^{13}C NMR (D_2O) δ 179.0, 175.6, 171.1 (C=O), 75.2, 75.0, 68.2, 67.6, 67.1 (C-2,4,5,6,7), 50.9, 49.8 (CH), 43.3 (C-8), 29.4, (C-3), 17.7, 17.1 (CH_3); FAB-MS, [M + H]$^+$ at *m/z* 364.1747 (calcd 364.1720). Anal. ($C_{14}H_{25}O_8N_3 \cdot 2H_2O$) C, H, N.

Methyl *N*-[*N*-(Benzyloxycarbonyl)-L-valyl-L-methionyl]-8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonate (10). *N*-Cbz-L-valyl-L-methionine (287 mg, 0.75 mmol), **6** (150 mg, 0.64 mmol), 1-hydroxybenzotriazole (70 mg, 0.5 mmol), triethylamine (97 μL, 0.7 mmol), and dicyclohexylcarbodiimide (165 mg, 0.8 mmol) were dissolved in DMF/2-propanol (10:1) (5.5 mL), and the mixture was stirred at room temperature overnight. Concentration and purification on silica gel with $CHCl_3$/MeOH (10:1) afforded **10** (75 mg, 20%): R_f 0.4 ($CHCl_3$/MeOH, 8:1); ^{13}C NMR (DMF-d_7) δ 173.3, 172.6, 172.5, 157.5 (C=O), 138.1, 129.1, 128.5, 128.4 (aromatic), 77.2, 73.2, 68.7, 67.4, 67.3, 66.7 (C-2,4,5,6,7, CH_2Ph), 61.6 (CH-Val), 53.2 (CH-Met), 52.3 (OCH_3), 44.0 (C-8), 34.3, 32.7, 30.7, 29.6 (CH-Val, 2 CH_2-Met, C-3), 19.7, 18.3 (2 CH_3-Val), 15.0 (SCH_3).

***N*-(L-Valyl-L-methionyl)-8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonic Acid (18).** Ester hydrolysis of **10** (96 mg, 0.16 mmol) was performed in H_2O/THF (1:1) (6 mL) followed by ion exchange in water according to general procedure A and hydrogenation was performed in water (5 mL) according to general procedure B. This afforded **18** as an amorphous solid (50 mg, 68%): R_f 0.65 (2-propanol/H_2O, 3:1, single spot with ninhydrin); $[\alpha]^{20}_D$ +53.2° (*c* 0.3, H_2O); ^{13}C NMR (D_2O) δ 179.1, 174.3, 171.5 (C=O), 75.4, 75.1, 68.2, 67.7, 67.2 (C-2,4,5,6,7), 59.7 (CH-Val), 54.6 (CH-Met), 43.5 (C-8), 31.5, 31.1, 30.2, 29.5, (CH-Val, 2 CH_2-Met, C-3), 18.8, 17.6 (2 CH_3-Val), 15.1 (SCH_3); FAB-MS, [M + H]$^+$ at *m/z* 452.2027 (calcd 452.2067).

***N*-(Benzyloxycarbonyl)-L-prolyl-L-methionine Succinimido Ester.** This compound was prepared according to general procedure C from *N*-hydroxysuccinimide (0.15 g, 1.3 mmol) and *N*-Cbz-L-prolyl-L-methionine (0.5 g, 1.3 mmol). Precipitation from MeOH/diethyl ether gave the desired compound (0.56 g, 90%): R_f 0.5 (EtOAc); ^{13}C NMR ($CDCl_3$) δ 168.8, 167.5, 157.7 (C=O), 136.4, 128.5, 128.1, 127.9 (aromatic), 67.4 (CH_2Ph), 60.3 (CH-Pro), 50.0 (CH-Met), 47.3 (CH_2-Pro), 31.5, 29.7, 28.0 (2 CH_2-Pro, CH_2-Met), 25.6 (2 CH_2-OSu), 24.9 (CH_2-Opro), 15.4 (SCH_3).

Methyl *N*-[*N*-(Benzyloxycarbonyl)-L-prolyl-L-methionyl]-8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonate (11). Compound **6** (0.27 g, 1.15 mmol), the above *N*-Cbz-L-prolyl-L-methionine succinimido ester (0.56 g, 1.17 mmol), and triethylamine (160 μL, 1.17 mmol) were dissolved in DMF (10 mL), and the mixture was stirred at room temperature for 1 h. Concentration and purification on silica gel with $CHCl_3$/MeOH (9:1) afforded **11** (0.36 g, 51%): R_f 0.6 ($CHCl_3$/MeOH, 4:1); ^{13}C NMR ($CDCl_3$) δ 172.9, 172.8, 172.5, 156.1 (C=O), 136.3,

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128.6, 128.1, 127.8 (aromatic), 75.5, 72.6, 68.6, 67.5, 66.6 (C-2,4,5,6,7), 66.7 (CH_2Ph), 61.0 (CH-Pro), 52.9 (CH-Met), 52.4, (OCH_3), 47.3 (CH_2-Pro), 43.5 (C-8), 31.7, 30.5, 30.5, 29.0 (2 CH_2-Met, CH_2-Pro, C-3), 24–6 (CH_2-Pro), 15.4 (SCH_3).

***N*-(L-Prolyl-L-methionyl)-8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonic Acid (19).** The methyl ester 11 (0.33 g, 0.55 mmol) was hydrolyzed in H_2O/THF/DMF (7:6:2) (15 mL) according to general procedure A (ion exchange with H_2O/THF (1:2) as eluent) and hydrogenation was performed in H_2O/THF (2:1) (3 mL) according to general procedure B. This afforded **19** as an amorphous solid (0.15 g, 57%): R_f 0.57 (2-propanol/H_2O (3:2), weak additional spots at R_f 0.35 and 0.49 with ninhydrin and sulfuric acid; these minor impurities do not give rise to additional peaks in the ^{13}C NMR spectrum); $[\alpha]^{20}_D$ +27.7° (*c* 0.8, H_2O); ^{13}C NMR (D_2O) δ 179.0, 174.2, 170.3 (C=O), 75.4, 75.1, 68.1, 67.7, 67.2 (C-2,4,5,6,7), 60.6 (CH-Pro), 54.5 (CH-Met), 47.3 (CH_2-Pro), 43.5 (C-8), 31.4, 30.3, 30.2, (2 CH_2-Met, CH_2-Pro), 29.4 (C-3), 24.5 (CH_2-Pro), 15.0 (SCH_3); FAB-MS, $[M - H]^-$ at *m/z* 448.1832 (calcd 448.1754).

Methyl *N*-L-Norvalyl-8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonate. *N*-Cbz-L-norvaline succinimido ester[21b] (1.2 g, 3.4 mmol), prepared according to general procedure C, and **6** (0.68 g, 2.9 mmol) were dissolved in THF/MeOH (1:1) (16 mL), and the mixture was stirred at room temperature for 2 h. Concentration and precipitation of the product from MeOH/diethyl ether was followed by hydrogenation in methanol (13 mL) according to general procedure B. This afforded the title compound (0.50 g, 44%): R_f 0.64 (MeOH/triethylamine, 9:1); ^{13}C NMR (DMF-d_7) δ 174.0, 173.1 (C=O), 77.1, 73.0, 69.0, 67.4, 67.2 (C-2,4,5,6,7), 55.6 (CH), 52.2 (OCH_3), 43.6 (C-8), 38.0 (CH_2), 29.6 (C-3), 19.4 (CH_2), 14.2 (CH_3).

Methyl *N*-(*N*$^{\alpha}$,*N*^{G},*N*^{G}-Tris(benzyloxycarbonyl)-L-arginyl-L-norvalyl-8-amino-2,6-anhydro-3,8-dideoxy-*glycero*-D-*talo*-octonate (12). *N*$^{\alpha}$,*N*^{G},*N*^{G}-Tri-Cbz-L-arginine succinimido ester (0.6 g, 0.9 mmol) and the above methyl *N*-L-norvalyl-8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonate (0.3 g, 0.9 mmol) were dissolved in THF/MeOH (1:1) (40 mL), and the mixture was stirred at room temperature for 2 h. Concentration and purification on silica gel with $CHCl_3$/MeOH (20:1) followed by 1:1 afforded **12** (0.39 g, 49%): mp 155–160 °C; R_f 0.39 ($CHCl_3$/MeOH, 8:1); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 173.9, 173.2, 173.0 (C=O), 163.9, 161.0, 157.1, 156.0 (C-Arg), 136.9, 135.4, 129.1, 128.7, 128.6, 128.4, 128.2, 128.1 (aromatic), 75.6, 72.8, 69.3, 68.7, 67.5, 67.3, 66.8, 66.7 (C-2,4,5,6,7,3 CH_2Ph), 55.4, 53.7 (CH), 52.6 (OCH_3), 44.7, 43.2 (C-8, CH_2-Arg), 34.5 (CH_2-Nva), 28.9 (C-3), 28.9, 25.4 (CH_2-Arg), 19.1 (CH_2-Nva), 13.7 (CH_3).

***N*-(L-Arginyl-L-norvalyl)-8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonic Acid (20).** Ester hydrolysis of **12** (0.35 g, 0.39 mmol) in THF/H_2O (1:1) (20 mL) according to general procedure A (ion exchange with H_2O/THF (4:1) as eluent) followed by hydrogenation in water (25 mL) according to general procedure B afforded **20** as an amorphous solid (0.15 g, 81%): R_f 0.25 (1-butanol/acetic acid/H_2O, 2:1:1, single spot with ninhydrin and sulfuric acid); $[\alpha]^{20}_D$ +43.4° (*c* 1.9, H_2O); ^{13}C NMR (D_2O) δ 179.1, 177.1, 175.6 (C=O), 157.6 (C-Arg), 75.4, 75.1, 68.2, 67.7, 67.2 (C-2,4,5,6,7), 55.1, 54.6 (CH), 43.4 (C-8), 41.6 (CH_2-Arg), 34.2 (CH_2-Nva), 31.7 (CH_2-Arg), 29.5 (C-3), 25.0 (CH_2-Arg), 19.4 (CH_2-Nva), 13.8 (CH_3); FAB-MS, $[M + H]^+$ at *m/z* 477.2720 (calcd 477.2673). Anal. ($C_{19}H_{36}N_6O_8 \cdot 2H_2O$) C, H, N 15.6 (calcd 16.4).

Methyl *N*-[*N*-(Benzyloxycarbonyl)-L-alanyl-L-phenylalanyl]-8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonate (13). *N*-Cbz-L-alanyl-L-phenylalanine succinimido ester[27] (0.60 g, 1.3 mmol), prepared according to general procedure C, **6** (0.27 g, 1.1 mmol), and triethylamine (176 μL, 1.3 mmol) were dissolved in MeOH/THF (10:1) (11 mL), and the mixture was stirred at room temperature for 3 h. Concentration and purification on silica gel with $CHCl_3$/MeOH (8:1) afforded **13** (0.21 g, 31%): R_f 0.35 ($CHCl_3$/MeOH, 8:1); ^{13}C NMR (DMF-d_7) δ 173.2, 173.1, 172.0, 156.9 (C=O), 138.7, 137.9, 130.0, 129.0, 128.7, 128.4, 126.8 (aromatic), 77.1, 73.0, 68.8, 67.3, 67.3, 66.5 (C-2,4,5,6,7, CH_2Ph), 55.0 (CH-Phe), 52.2 (OCH_3), 51.7 (CH-Ala), 44.2 (C-8), 38.4 (CH_2Ph), 29.6 (C-3), 18.3 (CH_3).

N-(L-Alanyl-L-phenylalanyl)-8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonic Acid (21). The ester **13** (0.21 g, 0.36 mmol) was hydrolyzed in H_2O/THF/DMF (5:3:3) (11 mL) according to general procedure A (ion exchange with water as eluent) and hydrogenation was performed in H_2O/THF (1:1) (14 mL) according to general procedure B. This afforded **21** as an amorphous solid (0.16 g, 100%): R_f 0.67 (2-propanol/H_2O, 3:1, single spot with ninhydrin); $[\alpha]^{20}_D$ +64.1° (*c* 0.4, H_2O); ^{13}C NMR (D_2O) δ 179.0, 174.0, 171.4 (C=O), 137.1, 130.3, 129.5, 127.5 (aromatic), 75.1, 74.9, 67.9, 67.7, 67.1 (C-2,4,5,6,7), 56.5 (CH-Phe), 49.9 (CH-Ala), 43.5 (C-8), 38.0 (CH_2Ph), 29.5 (C-3), 17.2 (CH_3); FAB-MS, $[M - H]^-$ at *m/z* 438.1826 (calcd 438.1877).

***N*$^{\alpha}$,*N*$^{\epsilon}$-Bis(benzyloxycarbonyl)-L-lysyl-L-leucine Succinimido Ester.** The title compound was synthesized from *N*-hydroxysuccinimide (0.22 g, 1.9 mmol) and *N*$^{\alpha}$,*N*$^{\epsilon}$-bis(benzyloxycarbonyl)-L-lysyl-L-leucine (1.0 g, 1.9 mmol) according to general procedure C. This afforded the desired compound (1.1 g, 90%): R_f 0.63 (EtOAc); ^{13}C NMR ($CDCl_3$) δ 172.7, 169.3, 168.0, 156.9, 156.5 (C=O), 136.6, 136.3, 128.4, 128.0, 127.9 (aromatic), 67.0, 66.6 (CH_2Ph), 54.7, 51.0 (CH), 40.6, 40.3 (CH_2-Leu, CH_2-Lys), 29.2 (CH_2-Lys), 25.5 (CH_2-Leu), 24.8 (CH-Leu), 22.8 (CH_3-Leu), 22.3 (CH_2-Lys), 21.7 (CH_3-Leu).

Methyl *N*-[*N*$^{\alpha}$,*N*$^{\epsilon}$-Bis(benzyloxycarbonyl)-L-lysyl-L-leucyl]-8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonate (14). The above *N*$^{\alpha}$,*N*$^{\epsilon}$-di-Cbz-L-lysyl-L-leucine succinimido ester (0.57 g, 0.9 mmol), **6** (0.19 g, 0.8 mmol), and triethylamine (125 μL, 0.9 mmol) were dissolved in methanol (8 mL). After 2 h at room temperature the solvent was evaporated in vacuo and purification on silica gel with $CHCl_3$/MeOH (8:1) afforded **14** (0.31 g, 51%): R_f 0.36 ($CHCl_3$/MeOH, 8:1); ^{13}C NMR (DMF-d_7) δ 173.2, 173.1, 172.8, 157.1 (C=O), 138.3, 137.9, 129.0, 128.3 (aromatic), 77.1, 73.0, 68.8, 67.3, 67.2, 66.4, 66.0 (C-2,4,5,6,7, 2 CH_2Ph), 57.3, (CH), 56.1 (CH), 52.2 (OCH_3), 44.0 (C-8), 41.7, 41.1 (CH_2-Leu, CH_2-Lys), 30.1, 29.8, 29.5, 29.4 (C-3, 2 CH_2-Leu, CH-Leu), 25.1, 23.4, 21.8 2 (2 CH_3-Leu, CH_2-Lys).

***N*-(L-Lysyl-L-leucyl)-8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonic Acid (22).** Compound **14** (0.30 g, 0.4 mmol) was hydrolyzed in THF/H_2O (5:2) (14 mL) according to general procedure A (ion exchange with THF/H_2O (1:1) as eluent) and hydrogenation was performed in THF/H_2O (1:1) (12 mL) according to general procedure B. This gave **22** as an amorphous solid (0.14 g, 76%): R_f 0.30 (1-butanol/acetic acid/H_2O, 2:1:1, weak additional spot at R_f 0.45 visible with ninhydrin but not with sulfuric acid); $[\alpha]^{20}_D$ +42.0° (*c* 0.8, H_2O); ^{13}C NMR (D_2O) δ 179.1, 176.1, 176.0 (C=O), 75.5, 75.1, 68.3, 67.7, 67.2, (C-2,4,5,6,7), 54.6 (CH), 54.0 (CH), 43.5 (C-8), 41.0, 40.1 (CH_2-Leu, CH_2-Lys), 33.5, 29.5, 27.4, (C-3, 2 CH_2-Leu), 25.3 (CH-Leu), 22.5 (CH_2-Lys), 23.0, 21.8 (CH_3-Leu); FAB-MS, $[M - H]^-$ at *m/z* 461.2645 (calcd 461.2611).

Determination of Minimum Inhibitory Concentration (MIC). Twofold serial dilutions of the test compound were made in Goldman and Leive[28] defined minimal medium in the wells of a Microtitre plate, giving a final volume of 100 μL/well. Bacterial (10^3), from an overnight bacterial culture, were inoculated into each well, and the Microtitre plate was incubated at 37 °C overnight. The MIC was judged to be the lowest drug concentration at which no turbidity could then be detected.

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Registry No. **4**, 106174-63-4; **5**, 106174-79-2; **6**, 106174-54-3; **7**, 110512-39-5; **8**, 110512-40-8; **9**, 110512-41-9; **10**, 110512-42-0; **11**, 110512-43-1; **12**, 110512-44-2; **13**, 110512-45-3; **14**, 110512-46-4; **15**, 110512-47-5; **16**, 110512-48-6; **17**, 110347-66-5; **18**, 110512-49-7; **19**, 110347-67-6; **20**, 110512-50-0; **21**, 110512-51-1; **22**, 110512-52-2; Cbz-Ala-OSu, 3401-36-3; Cbz-Met-OSu, 3392-01-6; Cbz-Ala-Ala-OSu, 16946-96-6; Cbz-Val-Met-OH, 108543-82-4; Cbz-Pro-Met-OH, 17730-18-6; Cbz-Pro-Met-OSu, 110529-59-4; Cbz-Nva-OSu, 71447-85-3; Cbz-Arg(Cbz$_2$)-OSu, 50715-13-4; Cbz-Ala-Phe-OSu, 20911-65-3; Cbz-Lys(Cbz)-Leu-OH, 13126-04-0; Cbz-Lys(Cbz)-Leu-OSu, 110512-54-4; methyl *N*-L-norvalyl-8-amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonate, 110512-53-3.

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