GG: mp 116–118 °C; ^{1}H NMR ($CDCl_3$) δ 1.82 (s, 3 H), 3.07 (s, 3 H), 3.37 (s, 3 H), 3.54 (m, 2 H), 3.77 (s, 2 H), 3.84 (m, 2 H), 5.35 (s, 2 H), 7.21 (s, 1 H), 7.32 (d, 1 H, J = 16), 7.43 (m, 2 H), 7.51 (d, 1 H, J = 9), 7.69 (d, 1 H, J = 9), 7.87 (m, 3 H).

Microbiological Evaluation of Compounds. The minimum inhibitory concentrations (MICs) were determined by using the 2-fold dilution method. The aerobic MIC values were determined on brain–heart infusion agar, and the anaerobic MIC values were determined with Wilkens-Chalgren broth. All values are expressed in micrograms/milliliter.

DNA Gyrase Supercoiling Inhibition Assay. The DNA gyrase supercoiling inhibition assay was performed by an agarose gel electrophoresis technique in a manner similar to that described previously.[12] Test compounds were dissolved in methanol (100%). The final concentration of methanol in each reaction mixture, including the control tube without the drug, was 12.5%. Methanol, at this concentration, does not affect the enzyme activity nor change the inhibitory potency of the norfloxacin control.

Registry No. **6** (R_1 = i-C_3H_7; R_2 = CH_3), 57691-99-3; **6** (R_1 = n-$C_{11}H_{23}$; R_2 = CH_3), 118895-40-2; **6** (R_1 = C_6H_{11}; R_2 = CH_3), 100764-16-7; **6** (R_1 = $C_6H_{11}CH_2$; R_2 = CH_3), 96227-85-9; **6** (R_1 = $C_6H_{11}CH(CH_3)$; R_2 = CH_3), 118895-41-3; **6** (R_1 = tetrahydropyran-2-yl; R_2 = CH_3), 118895-42-4; **6** (R_1 = p-$(CH_3)_2NC_6H_4$; R_2 = H), 20432-35-3; **6** (R_1 = o-$NO_2C_6H_4$; R_2 = H), 66894-06-2; **6** (R_1 = C_6H_5; R_2 = H), 14371-10-9; **6** (R_1 = C_6H_5; R_2 = Br), 33603-90-6; **6** (R_1 = C_6H_5; R_2 = Cl), 33603-89-3; **6** (R_1 = C_6H_5; R_2 = CH_3), 15174-47-7; **6** (R_1 = C_6H_5; R_2 = n-C_5H_{11}), 78605-96-6; **6** (R_1 = $C_6H_5CH(CH_3)$; R_2 = CH_3), 118895-41-3; **6** (R_1 = 2-C_5H_4N; R_2 = CH_3), 75102-17-9; **6** (R_1 = 1-$C_{10}H_7$; R_2 = CH_3), 75102-13-5; **6** (R_1 = 2-$C_{10}H_7$; R_2 = CH_3), 118895-36-6; **6** (R_1 = p-MeO-1-$C_{10}H_6$; R_2 = CH_3), 118895-43-5; **6** (R_1 = p-F-1-$C_{10}H_6$; R_2 = CH_3), 118895-44-6; **6** (R_1 = p-$C_6H_5C_6H_4$; R_2 = CH_3), 75102-04-4; **6** (R_1 = quinolin-4-yl; R_2 = CH_3), 118895-39-9; **6** (R_1 = quinolin-3-yl; R_2 = CH_3), 118895-45-7; **6** (R_1 = 1,2,3,4-tetrahydronaphthalen-2-yl; R_2 = CH_3), 118895-46-8; **6** (R_1 = o-HO-1-$C_{10}H_6$; R_2 = CH_3), 118895-47-9; **6** (R_1 = o-MEMO-1-$C_{10}H_6$; R_2 = CH_3), 118895-48-0; **7** (R_3 = CH_3; R_4 = H), 118895-32-2; **7** (R_3 = R_4 = CH_3), 118920-49-3; **7** (R_3, R_4 = $(CH_2)_3$), 118895-33-3; **7** (R_3 = 2,4-dimethoxybenzyl; R_4 = H), 95218-33-0; **8A**, 118894-99-8; **8AA**, 118895-24-2; **8B**, 118895-00-4; **8BB**, 118895-25-3; **8C**, 118895-01-5; **8CC**, 118895-26-4; **8D**, 118895-02-6; **8DD**, 118895-27-5; **8E**, 118895-03-7; **8EE**, 118895-28-6; **8F**, 118895-04-8; **8FF**, 118895-29-7; **8G**, 118895-05-9; **8GG**, 118895-30-0; **8H**, 118895-06-0; **8I**, 118895-07-1; **8J**, 118895-08-2; **8K**, 118895-09-3; **8L**, 118895-10-6; **8M**, 118895-11-7; **8N**, 118895-12-8; **8O**, 118895-13-9; **8P**, 118895-14-0; **8Q**, 118895-15-1; **8R**, 118920-48-2; **8S**, 118895-16-2; **8T**, 118895-17-3; **8U**, 118895-18-4; **8V**, 118895-19-5; **8W**, 118895-20-8; **8X**, 118895-21-9; **8Y**, 118895-22-0; **8Z**, 118895-23-1; **9**, 52148-44-4; **11** (R_3 = CH_3; R_4 = H; R_5 = CH_2CH_3), 118895-31-1; diketene, 674-82-8; sarcosine ethyl ester, 13200-60-7; N-methyl-D-alanine ethyl ester, 118895-34-4; D,L-proline methyl ester, 52183-82-1; 2-naphthaldehyde, 66-99-9; (carbethoxyethylidene)triphenylphosphorane, 54356-04-6; ethyl 3-naphth-2-ylmethacrylate, 100510-32-5; 2-methyl-3-naphth-2-ylprop-2-en-1-ol, 118895-35-5; 4-quinolinecarboxaldehyde, 4363-93-3; ethyl 3-quinolin-4-ylmethacrylate, 118895-37-7; 2-methyl-3-quinolin-4-ylprop-2-en-1-ol, 118895-38-8; RNA polymerase, 9014-24-8.

Synthesis of 8-Substituted Derivatives of the 2-Deoxy Analogue of 3-Deoxy-β-D-*manno*-2-octulopyranosonic Acid (2-Deoxy-β-KDO) as Inhibitors of 3-Deoxy-D-*manno*-octulosonate Cytidylyltransferase[1]

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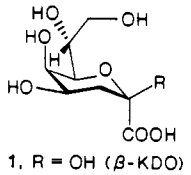
The 2-deoxy analogue of 3-deoxy-β-D-*manno*-2-octulopyranosonic acid (2-deoxy-β-KDO, **2**) is a potent inhibitor of the enzyme 3-deoxy-D-*manno*-octulosonate cytidylyltransferase, which is involved in the biosynthesis of lipopolysaccharide, an essential component of the outer membrane of Gram-negative bacteria. Since compound **2** lacks antibacterial activity, a series of 8-substituted derivatives of **2** has been synthesized in an attempt to find enzyme inhibitors suitable for modification as antibacterials. Compounds **9**, **11**, and **13**, in which the 8-hydroxy group of **2** is replaced by F, H, and NH_2, respectively, were as potent inhibitors of the enzyme as **2**, but were devoid of antibacterial activity, with the exception of the amino acid **13**, which showed weak activity against some strains of *Salmonella typhimurium*.

Recent work in these laboratories aimed at finding novel antibacterial agents has been focused on the design and synthesis of potential inhibitors of the biosynthesis of the lipopolysaccharide (LPS) of Gram-negative bacteria.[2-4] Enzymes involved in the incorporation of the sugar 3-deoxy-D-*manno*-2-octulosonic acid[5] (KDO, **1**) into LPS have been considered as attractive targets since bacterial mutants defective in KDO biosynthesis are not viable.[6]



1, R = OH (β-KDO)
2, R = H

KDO provides the link between the hydrophilic polysaccharide part of LPS and the hydrophobic lipid A moiety, an acylated and phosphorylated β-(1→6)-linked glucosamine disaccharide which is embedded in the outer bacterial membrane.[7] The enzyme 3-deoxy-D-*manno*-octulosonate cytidylyltransferase (CMP-KDO synthetase; EC 2.7.7.38) converts KDO (**1**) to the nucleotide sugar cytidine 5′-monophosphate KDO (CMP-KDO), which in turn serves as substrate for a series of KDO-lipid A

(1) A preliminary report of this work has been presented; see: Claesson, A.; Jansson, A. M.; Pring, B. G. *Carbohydrates 1987.* Abstracts of 4th European Carbohydrate Symposium, July 12–17, 1987, Darmstadt, FRG; Abstr. A-171.
(2) Molin, H.; Pring, B. G. *Tetrahedron Lett.* **1985**, *26*, 677.
(3) Claesson, A.; Luthman, K.; Gustafsson, K.; Bondesson, G. *Biochem. Biophys. Res. Commun.* **1987**, *143*, 1063.
(4) Luthman, K.; Claesson, A.; Jansson, A.; Pring, B. G. *Carbohydr. Res.* **1987**, *166*, 233.
(5) For a review, see: Unger, F. M. *Adv. Carbohydr. Chem. Biochem.* **1981**, *38*, 323.
(6) Rick, D.; Osborn, M. J. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 3756.
(7) Hammond, S. M. In *The Bacterial Cell Surface*; Hammond, S. M., Lambert, P. A., Rycroft, A. N., Eds.; Croom Helm: London, 1984; pp 57–82.

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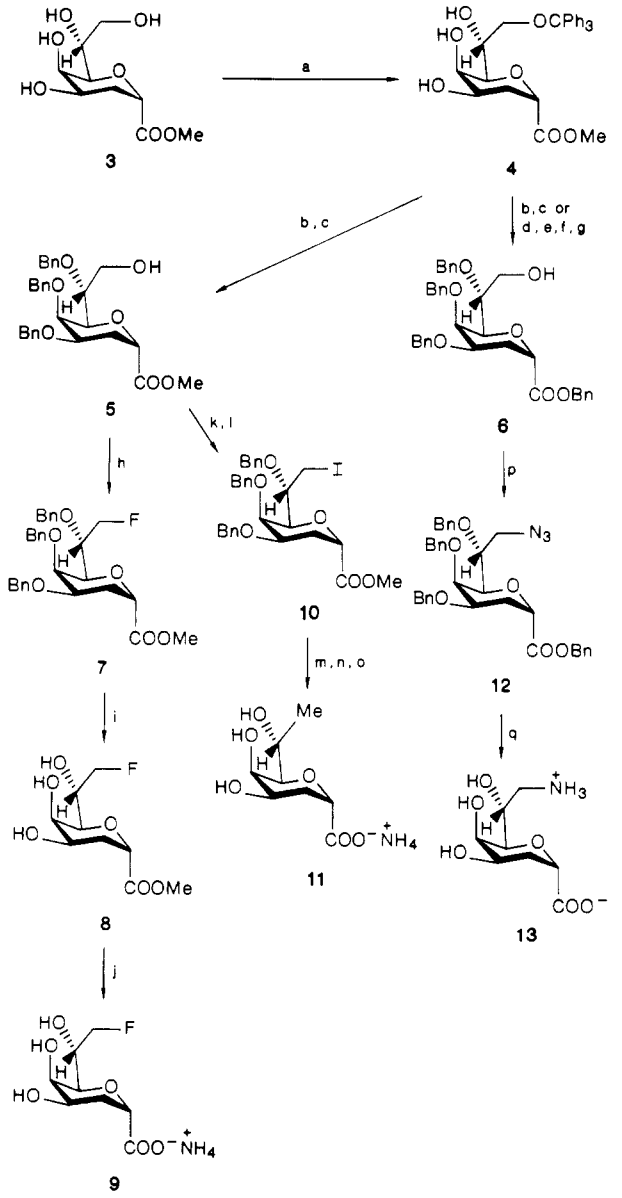
Scheme I[a]

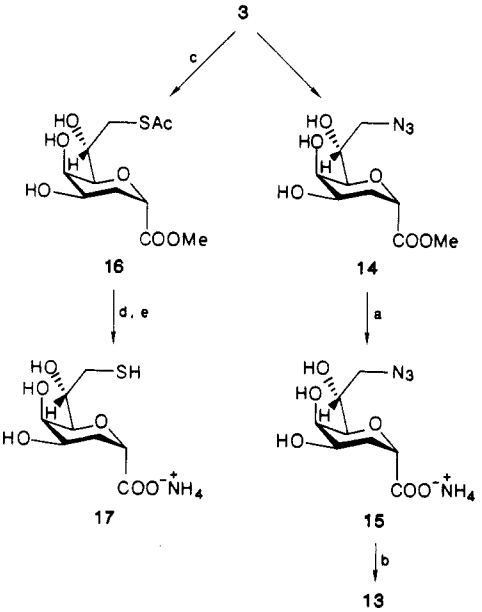


[a] Reagents: (a) Ph_3CCl, pyridine; (b) $PhCH_2Br$, Ag_2O, DMF; (c) H^+ resin, MeOH–$PhCH_3$; (d) LiOH, THF–H_2O; (e) pyridinium resin, PrOH–H_2O; (f) $PhCH_2Br$, NaH, Bu_4NI, DMF–THF; (g) AcOH–HCOOH–H_2O; (h) DAST, Et_3N, CH_2Cl_2; (i) H_2, Pd/C, MeOH–THF; (j) NaOH, H_2O, NH_4^+ resin; (k) *p*-TsCl, pyridine, Me_2CO; (l) NaI, DMF; (m) H_2, Pd/C, Et_3N, MeOH–THF; (n) H_2, Pd/C, MeOH–THF; (o) NaOH, H_2O, NH_4^+ resin; (p) LiN_3, CBr_4, Ph_3P, DMF; (q) H_2, Pd/C, EtOH–H_2O–THF.

transferases, able to incorporate KDO into the developing oligosaccharide.[8] Since the formation of CMP-KDO is thought to be the rate-limiting step in KDO incorporation,[9] the enzyme CMP-KDO synthetase constitutes a particularly attractive target. The β-pyranose form of KDO (β-KDO) has been shown by means of NMR studies to be the substrate for CMP-KDO.[10]

On testing structural analogues of KDO for inhibitory activity in an enzyme assay using CMP-KDO synthetase from *Escherichia coli* D 21, it was found that 2-deoxy-β-KDO (**2**) was a potent inhibitor of the enzyme (K_i = 3.9 μM).[3] However, this compound lacked antibacterial activity owing to its inability to cross the cytoplasmic membrane.[11a] Therefore, a series of 8-substituted derivatives of 2-deoxy-β-KDO was synthesized in an attempt to find compounds which may be more potent enzyme inhibitors and which may be more suitable for modification as potential transport forms to enable penetration into the bacterial cytoplasm.

Scheme II[a]



[a] Reagents: (a) NaOH, H_2O, NH_4^+ resin; (b) H_2, Pd/C, THF–H_2O, NH_4OH; (c) DEAD, Ph_3P, AcSH, THF–MeCN; (d) MeONa, MeOH; (e) NaOH, H_2O, H^+ resin, NH_4OH.

Chemistry

The 8-substituted derivatives of 2-deoxy-β-KDO were synthesized as depicted in Schemes I and II. Compounds **9** and **11** were obtained from the methyl ester of 2-deoxy-β-KDO[12] (**3**) via the 4,5,7-tri-*O*-benzyl derivative **5**. Thus fluorination of **5** with (diethylamido)sulfur trifluoride (DAST), followed by deprotection and ion exchange, afforded the 8-deoxy-8-fluoro derivative **9**, while conversion of **5** to the 8-tosylate followed by iodination, hydrogenation, saponification, and ion exchange afforded the 8-deoxy derivative **11**. The amino acid **13** could be obtained via the benzyl ester of the 4,5,7-tri-*O*-benzyl derivative of 2-deoxy-β-KDO (**6**), which was obtained as a byproduct in the synthesis of methyl ester **5** from compound **4**, the 8-*O*-trityl derivative of methyl ester **3**, by the benzyl bromide–Ag_2O method.[13] A more efficient synthesis of **6** was devised whereby **4** was subjected to ester hydrolysis and converted to the pyridinium salt, which was then benzylated with benzyl bromide–NaH–Bu_4NI.[14] Conversion of **6** to the 8-azido-8-deoxy derivative by the Ph_3P–CBr_4–LiN_3 method[15] followed by reduction–depro-

(8) Osborn, M. J. In *The Bacterial Outer Membrane*; Inouye, M., Ed.; Wiley: New York, 1979; pp 15–34.
(9) Ray, P. H.; Benedict, C. D.; Grasmuk, H. *J. Bacteriol.* **1981**, *145*, 1273.
(10) Kohlbrenner, W. E.; Fesik, S. W. *J. Biol. Chem.* **1985**, *260*, 14695.
(11) (a) Hammond, S. M.; Claesson, A.; Jansson, A. M.; Larsson, L.-G.; Pring, B. G.; Town, C. M.; Ekström, B. *Nature* **1987**, *327*, 730. (b) Goldman, R.; Kohlbrenner, W.; Lartey, P.; Pernet, A. *Nature* **1987**, *329*, 162.
(12) Luthman, K.; Orbe, M.; Wåglund, T.; Claesson, A. *J. Org. Chem.* **1987**, *52*, 3777.
(13) Kuhn, R.; Löw, I.; Trischmann, H. *Chem. Ber.* **1957**, *90*, 203.
(14) Czernecki, S.; Georgoulis, C.; Provelenghiou, C. *Tetrahedron Lett.* **1976**, 3535.
(15) Yamamoto, I.; Sekine, M.; Hata, T. *J. Chem. Soc., Perkin Trans. 1* **1980**, 306.

Table I. IC_{50} Values for 8-Substituted Derivatives of 2-Deoxy-β-KDO[a]

compd	IC_{50},[b] μM	
	CKS	CKS-KLT
2	18	10
9	24	4.8
11	22	6.8
13	c	7.4
15	c	80
17	c	17

[a] See text for a discussion of the merits of the two assays. [b] IC_{50} = concentration of test compound giving 50% inhibition. [c] The compound gave rise to a strongly interfering absorption in the thiobarbituric acid assay.

tection afforded **13**. An alternative and more direct route to **13** (Scheme II) was by hydrolysis of the 8-azido-8-deoxy derivative **14**,[16] to give **15** after ion exchange, followed by hydrogenation. The synthesis of **13** from **15** in this way has also been reported in a patent.[17] In order to obtain the 8-deoxy-8-thio derivative **17**, the methyl ester of 2-deoxy-β-KDO (**3**) was converted to the 8-acetylthio derivative **16** with Ph_3P–DEAD–AcSH[18] followed by deprotection in two steps.

Biology

The 8-substituted derivatives of 2-deoxy-β-KDO were tested as inhibitors of CMP-KDO synthetase from *Escherichia coli* D 21. The CMP-KDO synthetase (CKS) screening assay was based essentially on that described by Ghalambor and Heath,[19] the KDO liberated from the nucleotide being determined by a modified thiobarbituric acid assay.[20] However, certain derivatives (**13**, **15**, and **17**) gave rise to an interfering absorption in the colorimetric thiobarbituric acid assay. Therefore all the compounds were additionally tested in a combined CMP-KDO synthetase–KDO-lipid A transferase (CKS–KLT) assay, in which CMP-[1-^{14}C]KDO was generated in situ from CTP and [1-^{14}C]KDO[21] in the presence of CMP-KDO synthetase and incubated with lipid A precursor in the presence of KDO-lipid A transferase (cf. ref 22). The enzymes in this assay were prepared from a mutant of *Salmonella typhimurium* (SL 1102). The incorporation of [1-^{14}C]KDO into an acid-insoluble fraction was then determined with as well as without the test compound in order to ascertain the degree of enzyme inhibition. A 15-fold lower KDO concentration could be used in this assay than in the thiobarbiturate assay. A control assay was performed in which the test compounds were added subsequent to formation of CMP-KDO in a preincubation stage. There was no inhibition of KDO-lipid A transferase, thus showing that it is the CMP-KDO synthetase component of the mixture that is being inhibited in the combined CKS–KLT assay. This assay thus expresses a direct response to changes in the degree of inhibition of the CKS enzyme, and it must be considered as more reliable than the CKS assay based on the colorimetric method where interference from the inhibitor is possible. The IC_{50} values for the test compounds in both assays are given in Table I. Compounds **9**, **11**, and **13** were comparable in activity to 2-deoxy-β-KDO (**2**) as inhibitors of CMP-KDO synthetase, while compound **17** was somewhat less active than **2** and compound **15** was considerably less active. The compounds were also tested for antibacterial activity against *E. coli* ATCC 11303, *S. typhimurium* LT2M-1, *S. typhimurium* SL 1102, and *S. typhimurium* AG701i50. Compound **13** exhibited weak antibacterial activity (MIC 2 mM) against the two last-mentioned strains, whereas the other compounds tested were without activity at 2 mM.

Discussion

It has been inferred,[2] on the basis of NMR evidence that KDO is in the α-configuration when present in LPS,[23] and it has been subsequently confirmed[10] that the β-anomer of KDO is the natural substrate for CMP-KDO (i.e., transfer of KDO from CMP-KDO to the developing LPS occurs with inversion of configuration). This fact was also indicated by the observation that 2-deoxy-β-KDO (**2**) was a potent inhibitor of CMP-KDO synthetase, whereas 2-deoxy-α-KDO was inactive.[3] Compounds such as the carbocyclic analogue of β-KDO,[2] the azacyclic analogue of 2-deoxy-β-KDO,[24] analogues of 2-deoxy-β-KDO modified at the carboxylic group,[4] and an isosteric phosphonate analogue of CMP-KDO[25] have been reported as inferior to compound **2** as inhibitors of CMP-KDO synthetase.

The 8-substituted derivatives of 2-deoxy-β-KDO reported here were all potent inhibitors of CMP-KDO synthetase with the exception of the azido derivative **15** (see Table I). The inhibitory activity is independent of the electronic properties of the substituent since compounds **9**, **11**, and **13**, with F, H, and NH_2, respectively, at the 8-position were all similar to 2-deoxy-β-KDO (**2**) in inhibitory activity. However, steric effects appear to be important since compound **17**, which contains the somewhat larger thiol group, was somewhat less active than **2**, and compound **15** containing the linear azido group was much less active. Recently, values for the inhibitory activity of **2**, **13**, and **17** with a purified CMP-KDO synthetase enzyme have been reported.[26] These results are in agreement with our observation that **17** is less active than **2**, while compound **13** is reported as being a more effective inhibitor than **2**, and a chain-extended analogue of **13**, 8-(aminomethyl)-2,8-dideoxy-β-KDO, is reported as being even more active than **13**.[26] Of the compounds described here, only compound **13** showed limited antibacterial activity. However, by linking dipeptides to the amino terminal of **13**, it was possible to obtain derivatives with good antibacterial activity since they were actively transported across the cytoplasmic membrane and subsequently hydrolyzed to give the active inhibitor.[11,16]

Experimental Section

For the general experimental procedures, see ref 16. In addition, ^{1}H NMR spectra in D_2O were determined on a JEOL FX200 spectrometer using *tert*-butyl alcohol (δ = 1.23) as internal standard.

General Procedure for the Hydrolysis of the Methyl Esters. Hydrolysis was carried out with 0.5 M NaOH until completion according to TLC (20–40 min). The solution was then

(16) Claesson, A.; Jansson, A. M.; Pring, B. G.; Hammond, S. M.; Ekström, B. *J. Med. Chem.* **1987**, *30*, 2309.
(17) Rosenbrook, W.; Lartey, P. A.; Riley, D. A. U.S. Patent 4 613 590, 1986; *Chem. Abstr.* **1987**, *106*, 50600y.
(18) Jenkins, I. D.; Thang, S. *Aust. J. Chem.* **1984**, *37*, 1925.
(19) Ghalambor, M. A.; Heath, E. C. *J. Biol. Chem.* **1966**, *241*, 3216.
(20) Karkhanis, Y. D.; Zeltner, J. Y.; Jackson, J. J.; Carlo, D. J. *Anal. Biochem.* **1978**, *85*, 595.
(21) Bondesson, G.; Fagervall, I.; Gosztonyi, T.; Jansson, A.; Ohlberger, A. *J. Labelled Compd. Radiopharm.* **1982**, *19*, 239.
(22) Munson, R. S.; Rasmussen, N. S.; Osborn, M. J. *J. Biol. Chem.* **1978**, *253*, 1503.
(23) (a) Strain, S. M.; Fesik, S. W.; Armitage, I. M. *J. Biol. Chem.* **1983**, *258*, 2906. (b) Strain, S. M.; Fesik, S. W.; Armitage, I. M. *J. Biol. Chem.* **1983**, *258*, 13466.
(24) Norbeck, D. W.; Kramer, J. B. *Tetrahedron Lett.* **1987**, *28*, 773.
(25) Norbeck, D. W.; Kramer, J. B.; Lartey, P. A. *J. Org. Chem.* **1987**, *52*, 2174.
(26) Capobianco, J. O.; Darveau, R. P.; Goldman, R. C.; Lartey, P. A.; Pernet, A. G. *J. Bacteriol.* **1987**, *169*, 4030.

passed through an ammonium-saturated ion-exchange resin (Dowex 50 W × 8) with H_2O as eluent.

Methyl 2,6-Anhydro-3-deoxy-8-*O*-trityl-D-*glycero*-D-*talo*-octonate (4). Methyl 2,6-anhydro-3-deoxy-D-*glycero*-D-*talo*-octonate[12] (3) (12.8 g, 54.2 mmol) was tritylated with triphenylmethyl chloride (37.8 g, 135.5 mmol) in dry pyridine (200 mL) at 30 °C overnight. After conventional workup, the crude product was purified on silica gel with EtOAc–diisopropyl ether 3:2 to give 4 (20.4 g, 79%) as a semicrystalline residue: R_f 0.23 (EtOAc); $[\alpha]^{20}_D$ +29.3° (*c* 0.98, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 171.9 (C-1), 143.9, 128.7, 127.9, 127.8, 86.9 (trityl), 74.2, 72.4, 70.0, 67.2, 66.4 (C-2,4,5,6,7), 64.6 (C-8), 51.9 (OCH_3), 29.1 (C-3). Anal. ($C_{28}H_{30}O_7$) C, H.

Methyl 2,6-Anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-D-*glycero*-D-*talo*-octonate (5) and Benzyl 2,6-Anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-D-*glycero*-D-*talo*-octonate (6). Compound 4 (15 g, 31.4 mmol) was dissolved in dry DMF (130 mL), and benzyl bromide (32.2 g, 188 mmol) was added. The solution was cooled to −20 °C, and silver oxide (87.3 g, 377 mmol) was added in portions. After 30 min the temperature was raised to room temperature, and the solution was stirred for 3 days with protection from light. Methanol (7.6 mL) in DMF (40 mL) was then added dropwise at −35 °C. The mixture was slowly brought to room temperature, $CHCl_3$ (255 mL) was added, and the reaction mixture was filtered through hyflo and then concentrated. Purification of the product on silica gel with hexane–EtOAc 9:1 gave a mixture of the 8-*O*-trityl derivatives of 5 and 6 (8 g) which were directly detritylated in MeOH–toluene (740 mL, 10:1) containing IR 120 (H^+) (76 mL, prewashed with H_2O and MeOH). The reaction mixture was heated at 70 °C overnight, and after filtration and concentration, the residue was purified on silica gel with hexane–EtOAc 2:1 to afford 5 (2.95 g, 18%) as an oil: R_f 0.18 (hexane–EtOAc 3:2); $[\alpha]^{20}_D$ +23.6° (*c* 1.43, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 172.6 (C-1), 139.0, 138.3, 138.2 (C arom), 127.5–128.5 (CH arom), 77.0, 76.2, 74.7, 73.0, 72.3 (C-2,4,5,6,7), 74.2, 71.3, 70.5 (3 CH_2Ph), 60.5 (C-8), 52.5 (OCH_3), 27.0 (C-3). Anal. ($C_{30}H_{34}O_7$) C, H, O. Chromatography also afforded 6 (1.8 g, 10%): R_f 0.29 (EtOAc–hexane 3:2); $[\alpha]^{20}_D$ +8.6° (*c* 1.17, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 171.9 (C-1), 139.1, 138.4, 138.3, 135.4 (C arom), 127.4–128.7 (CH arom), 77.2, 76.4, 74.6, 72.9, 72.6 (C-2,4,5,6,7), 74.2, 71.3, 70.6, 67.3, 60.6 (4 CH_2Ph, C-8), 27.2 (C-3). Anal. ($C_{36}H_{38}O_7$) C, H, O.

Benzyl 2,6-Anhydro-4,5,7-tri-*O*-benzyl-3-deoxy-D-*glycero*-D-*talo*-octonate (6) (Alternative Synthesis Which Afforded 6 in High Yield.) Lithium hydroxide (0.6 g, 25 mmol) was added to a solution of 4 (5.0 g, 10 mmol) in THF–H_2O (50 mL, 3:1). After the mixture was stirred at room temperature for 1 h, TLC showed complete hydrolysis of the methyl ester, and the solution was passed through a pyridinium-saturated cation-exchange resin (Dowex 50 W × 8) with H_2O–2-propanol (3:1) as eluent. The eluate was concentrated to dryness, and the residue was dissolved in DMF–THF (100 mL, 1:1). Benzyl bromide (20.5 mL, 170 mmol) was added, and the solution was cooled to −10 °C. After introduction of sodium hydride (1.6 g, 40 mmol, 55–60% dispersion in oil) and tetrabutylammonium iodide (7.4 g, 20 mmol), the reaction mixture was stirred for 7 min, and then an additional amount of sodium hydride (2.1 g, 53 mmol) was added. After 4 h the reaction mixture was brought to room temperature and was stirred overnight. Triethylamine (10 mL) and triethylammonium chloride (3 g) were added, followed after 2 h by Et_2O and toluene (50 mL, 1:1), whereupon the reaction mixture was filtered through a small bed of silica gel. The solvent was evaporated, and the residue was purified on silica gel with petroleum ether–EtOAc (11:1) which gave the 8-*O*-trityl derivative of 6. Detritylation was performed in HOAc–HCOOH–H_2O (88 mL, 1:1:0.1) at room temperature for 20 min. Concentration and purification on silica gel with EtOAc–hexane 1:3 and 1:1 gave 6 (3.9 g, yield 67% from 4).

Methyl 2,6-Anhydro-4,5,7-tri-*O*-benzyl-3,8-dideoxy-8-fluoro-D-*glycero*-D-*talo*-octonate (7). A solution of 5 (730 mg, 1.4 mmol) and triethylamine (0.40 mL, 2.9 mmol) in CH_2Cl_2 (4 mL) was added dropwise to a solution of (diethylamido)sulfur trifluoride (0.35 mL, 2.9 mmol) in CH_2Cl_2 (3 mL) at −40 °C under a N_2 atmosphere. The mixture was slowly brought to room temperature. After 1 h the reaction mixture was diluted with CH_2Cl_2 (20 mL), and 6% aqueous $NaHCO_3$ (40 mL) was added dropwise. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (×2). The combined organic phases were washed with H_2O (×3) and dried ($MgSO_4$). Chromatography on silica gel with hexane–EtOAc 5:1 gave 7 (305 mg, 42%) as white crystals: mp 60–62 °C; R_f 0.52 (hexane–EtOAc 3:2); $[\alpha]^{20}_D$ +23° (*c* 1.04, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 172.0 (C-1), 138.9, 138.1 (C arom), 127.4–128.4 (CH arom), 83.6 (d, C-8, $^1J_{C-F}$ = 170 Hz), 76.4, 76.0, 73.6, 72.9, 72.4, (C-2,4,5,6,7), 74.2, 72.4, 70.5 (3 CH_2Ph), 52.1 (OCH_3), 27.0 (C-3). Anal. ($C_{30}H_{33}FO_6$) C, H, F.

Methyl 2,6-Anhydro-3,8-dideoxy-8-fluoro-D-*glycero*-D-*talo*-octonate (8). Compound 7 (280 mg, 0.55 mmol) was debenzylated by catalytic hydrogenation at 4 bar in MeOH–THF (24 mL, 11:1) with prehydrogenated 5% Pd/C (479 mg) as catalyst. An additional amount of the catalyst (200 mg) was added after 5 h, and after another 24 h the catalyst was filtered and washed with MeOH. Purification on silica gel with EtOAc–MeOH–toluene 7:2:1 gave 8 (74 mg, 56%): R_f 0.41 (EtOAc–MeOH–toluene 7:2:1); $[\alpha]^{20}_D$ +73.8° (*c* 0.52, MeOH); ^{13}C NMR (CD_3OD) δ 173.7 (C-1), 86.4 (d, C-8, $^1J_{C-F}$ = 167 Hz), 75.4 (d, C-6, $^3J_{C-F}$ = 7 Hz), 69.8 (d, C-7, $^2J_{C-F}$ = 18 Hz), 74.0, 68.0, 68.0 (C-2,4,5), 52.7 (OCH_3), 29.8 (C-3); FAB-MS, $(M - H)^-$ at *m/z* 237.0777 (calcd 237.0774). Anal. ($C_9H_{15}FO_6$) C, H; F: calcd, 7.97; found, 7.44.

Ammonium 2,6-Anhydro-3,8-dideoxy-8-fluoro-D-*glycero*-D-*talo*-octonate (9). Compound 8 (59 mg, 0.25 mmol) was hydrolyzed according to the general procedure. The eluate was concentrated and the residue was recrystallized from H_2O–EtOH which afforded 9 (52 mg, 87%): mp 147–149 °C; $[\alpha]^{20}_D$ +66° (*c* 1.06, H_2O); ^{13}C NMR (D_2O) δ 178.9 (C-1), 86.2 (d, C-8, $^1J_{C-F}$ = 165 Hz), 73.6 (d, C-6, $^3J_{C-F}$ = 7 Hz), 69.2 (d, C-7, $^2J_{C-F}$ = 17 Hz), 75.1, 67.6, 67.5 (C-2,4,5), 29.5 (C-3); ^1H NMR (D_2O) δ 5.0–4.4 (m, 3 H, H-7,8), 4.34 (d, 1 H, *J* = 6 Hz, H-2), 4.1–3.8 (m, 3 H, H-4,5,6), 2.3–1.9 (m, 2 H, H-3a,3e); FAB-MS, $(M - H)^-$ at *m/z* 223.0586 (calcd 223.0618). Anal. ($C_8H_{16}FNO_6 \cdot 0.33H_2O$) C, H, N; F: calcd, 7.68; found, 7.01.

Methyl 2,6-Anhydro-4,5,7-tri-*O*-benzyl-3,8-dideoxy-8-iodo-D-*glycero*-D-*talo*-octonate (10). Tosylation of 5 (1.1 g, 2.1 mmol) was carried out with *p*-toluenesulfonyl chloride (800 mg, 4.2 mmol) in acetone (0.7 mL) and pyridine (0.4 mL) with stirring at room temperature overnight. After workup, the crude product was purified on silica gel with toluene–EtOAc 20:1 to afford the 8-*O*-tosyl compound (1.3 g, 93%): R_f 0.3 (diisopropyl ether–hexane 4:1); $[\alpha]^{20}_D$ +11° (*c* 1.3, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 171.9 (C-1), 144.5, 138.9, 138.1, 137.7 (C arom), 127.4–129.7 (CH arom), 75.9, 75.0, 74.1, 73.8, 72.9, 72.4, 72.2, 70.4, 69.2 (C-2,4,5,6,7,8, 3 CH_2Ph), 52.1 (OCH_3), 26.9 (C-3), 21.5 (CH_3 arom). The tosylate (200 mg, 0.3 mmol) was dissolved in dry DMF (0.6 mL) and sodium iodide (114 mg, 0.76 mmol) was added. The solution was heated at 85 °C for 2.5 h, and after cooling to room temperature it was added dropwise to a mixture of H_2O and EtOAc (30 mL, 1:1). After phase separation the aqueous phase was extracted with EtOAc (15 mL), and the combined EtOAc phases were washed with 5% sodium thiosulfate (15 mL), and H_2O (2 × 7 mL). Drying (Na_2SO_4), filtration, and concentration gave 10 as a light yellow oil (165 mg, 88%): R_f 0.43 (diisopropyl ether–hexane 4:1); $[\alpha]^{20}_D$ −6° (*c* 1.5, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 171.6 (C-1), 139.1, 138.3, 137.5 (C arom), 127.4–128.4 (CH arom), 76.4, 75.9, 74.0, 73.1, 72.6 (C-2,4,5,6,7), 74.3, 71.1, 70.5 (3 CH_2Ph), 52.2 (OCH_3), 26.9 (C-3), 9.5 (C-8): FAB-MS, $(M + H)^+$ at *m/z* 617.1437 (calcd 617.1400).

Ammonium 2,6-Anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonate (11). Reduction of 10 (160 mg, 0.26 mmol) was performed in two steps. After hydrogenation overnight in MeOH–THF (4 mL, 3:1) and triethylamine (26 mg, 0.26 mmol) with 5% Pd/C (160 mg), an additional amount of the catalyst (70 mg) was added, and the hydrogenation was continued for another 24 h. The catalyst was filtered, and the solvent was evaporated. The residue was dissolved in EtOAc (30 mL) and washed with H_2O (15 mL), 1 N HCl (15 mL), and H_2O (15 mL). Drying of the extract and concentration quantitatively gave the benzylated 8-deoxy compound: R_f 0.37 (diisopropyl ether–hexane 4:1). This compound was then debenzylated by hydrogenation overnight in MeOH–THF (4 mL, 3:1) with 5% Pd/C (150 mg). An additional amount of catalyst (65 mg) was added and hydrogenation was continued over another night. The catalyst was filtered and, the solvent was evaporated. Purification on silica gel with EtOAc–MeOH–toluene 7:2:1 gave the methyl ester of compound 11 (15 mg, 26%): R_f 0.21 (EtOAc–MeOH–toluene 7:2:1) $[\alpha]^{20}_D$ +73°

(*c* 0.83, MeOH); ^{13}C NMR (CD_3OD) δ 174.0 (C-1), 80.3, 74.1, 68.5, 68.3, 67.1 (C-2,4,5,6,7), 52.7 (OCH_3), 30.0 (C-3), 20.9 (C-8). This ester was then hydrolyzed according to the general procedure. The eluate was concentrated and gave quantitatively **11**: $[\alpha]^{20}_D$ +57° (*c* 0.7, MeOH); ^{13}C NMR (D_2O) δ 179.1 (C-1), 78.3, 75.1, 67.9, 67.8, 67.1 (C-2,4,5,6,7), 29.6 (C-3), 20.2 (C-8); ^1H NMR (D_2O) δ 4.33 (dd, 1 H, *J* = 5.6 Hz, 1.5 Hz, H-2), 3.99 (d, 1 H, *J* = 2.5 Hz, H-5), 3.90 (dq, 1 H, *J* = 8 Hz, 6.5 Hz, H-7), 3.8–3.6 (m, 1 H, H-4), 3.47 (d, 1 H, *J* = 8 Hz, H-6), 2.16 (dd, 1 H, *J* = 12.5 Hz, 5 Hz, H-3*e*), 2.00 (ddd, 1 H, *J* = 12.5 Hz, 12.5 Hz, 6.5 Hz, H-3a), 1.29 (d, 3 H, *J* = 6.5 Hz, H-8); FAB-MS, $(M - H)^-$ at *m/z* 205.0722 (calcd 205.0712).

Benzyl 2,6-Anhydro-8-azido-4,5,7-tri-*O*-benzyl-3,8-dideoxy-D-*glycero*-D-*talo*-octonate (12). Triphenylphosphine (0.42 g, 1.6 mmol) was added to a solution of **6** (0.62 g, 1.1 mmol), lithium azide (0.37 g, 7.6 mmol), and tetrabromomethane (0.53 g, 1.6 mmol) in DMF (10 mL) at 0 °C under dry conditions, and the mixture was stirred at room temperature overnight. The solvent was evaporated, Et_2O was added to the residue, and the salts were filtered. Concentration and purification on silica gel with EtOAc–toluene 1:30 gave **12** (0.42 g, 76%): mp 50–51 °C (after recrystallization from Et_2O–petroleum ether); R_f 0.58 (diisopropyl ether/hexane 4:1); $[\alpha]^{20}_D$ +16° (*c* 1.6, $CHCl_3$); IR 2100 (azide), 1730 (ester) cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 171.2 (C-1), 138.9, 138.2, 137.8, 135.5 (C arom), 127.3–128.6 (CH arom), 76.5, 76.3, 74.6, 72.9, 72.6 (C-2,4,5,6,7), 74.1, 72.4, 70.5, 66.9 (4 CH_2Ph), 51.1 (C-8), 27.1 (C-3). Anal. ($C_{36}H_{37}N_3O_6$) C, H, N.

Ammonium 8-Azido-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonate (15). Methyl 2,6-anhydro-8-azido-3,8-dideoxy-D-*glycero*-D-*talo*-octonate[16] (**14**) (100 mg, 0.38 mmol) was hydrolyzed according to the general procedure. The eluate was freeze-dried to afford **15** as a white hygroscopic powder (92 mg, 90%): $[\alpha]^{20}_D$ +50.3° (*c* 0.6, H_2O); IR (KBr) 2105 cm^{-1} (azide); ^{13}C NMR (D_2O) δ 178.7 (C-1), 75.2, 75.0, 69.6, 67.6, 67.4 (C-2,4,5,6,7), 54.9 (C-8), 29.4 (C-3); ^1H NMR (D_2O) δ 4.32 (d, 1 H, *J* = 6 Hz, H-2), 4.0–3.8 (m, 2 H, H-5,7) 3.8–3.5 (m, 3 H, H-4,6,8), 3.45 (dd, 1 H, *J* = 13.5 Hz, 7.5 Hz, H-8′), 2.16 (dd, 1 H, *J* = 12.5 Hz, 4.5 Hz, H-3e), 1.98 (ddd, 1 H, *J* = 12.5 Hz, 12.5 Hz, 6 Hz, H-3a); FAB-MS, $(M - H)^-$ at *m/z* 246.0678 (calcd 246.0726).

8-Amino-2,6-anhydro-3,8-dideoxy-D-*glycero*-D-*talo*-octonic Acid (13). (a) From Compound 12. Compound **12** (0.5 g, 0.68 mmol) was deprotected by catalytic hydrogenation overnight in THF–H_2O–EtOH 1:2:7 with 5% Pd/C (520 mg). The catalyst was filtered and the solvent was evaporated. The residue was purified on silica gel with 1-propanol–H_2O 7:3. Crystallization from H_2O–EtOH gave **13** (60 mg, 40%): mp 300 °C dec; R_f 0.45 (1-propanol–H_2O 3:2); $[\alpha]^{20}_D$ +56° (*c* 1.9, H_2O); ^{13}C NMR (D_2O) δ 179.3 (C-1), 75.3, 75.0, 67.6, 66.8, 65.4 (C-2,4,5,6,7), 44.2 (C-8), 29.2 (C-3); ^1H NMR (D_2O) δ 4.36 (d, 1 H, *J* = 7 Hz, H-2), 4.2–4.0 (m, 1 H, H-7), 3.96 (br s, 1 H, H-5), 3.8–3.6 (m, 1 H, H-4), 3.46 (d, 1 H, *J* = 9 Hz, H-6), 3.4–3.1 (m, 2 H, H-8), 2.22 (dd, 1 H, *J* = 12.5 Hz, 5 Hz, H-3*e*), 2.01 (ddd, 1 H, *J* = 12.5 Hz, 12.5 Hz, 7 Hz, H-3*a*. Anal. ($C_8H_{15}NO_6$) C, H, N.

(b) From Compound 15 (See Also Ref 17). Hydrogenolysis of **15** (1.4 g, 5.3 mmol) was performed in THF–H_2O (1:1, 20 mL) at 4 bar for 3 h with 10% Pd/C as catalyst. The catalyst was filtered and washed with H_2O and the eluate was concentrated. The residue was purified by ion-exchange chromatography (Dowex 50 W × 8, H^+) with water as eluent and then with 2 M NH_4OH. Concentration of appropriate fractions gave **13** (1.1 g, 94%).

Methyl 8-*S*-Acetyl-2,6-anhydro-3,8-dideoxy-8-thio-D-*glycero*-D-*talo*-octonate (16). Triphenylphosphine (550 mg, 2.1 mmol) was dissolved in THF (5 mL). Diethyl azodicarboxylate (325 μL, 2.1 mmol) was added at 0 °C and when the phosphonium salt had solidified a solution of **3** (249 mg, 1.05 mmol) and thioacetic acid (150 μL, 2.1 mmol) in THF–CH_3CN (5 mL, 3:2) was added. Stirring at 0 °C for 10 min and at room temperature for 1 h gave a clear solution. Concentration and purification on silica gel with CH_2Cl_2–MeOH 19:1 gave **16** (170 mg, 55%): mp 115–117 °C; R_f 0.57 (EtOAc–MeOH–toluene 7:2:1); $[\alpha]^{20}_D$ +82.1° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 197.8 (SAc), 171.9 (C-1), 76.0, 72.5, 69.2, 66.9, 66.6 (C-2,4,5,6,7), 52.1 (OCH_3), 33.5 (C-8), 30.5 (SAc), 28.8 (C-3); FAB-MS, $(M - H)^-$ at *m/z* 293.0678 (calcd 293.0695). Anal. ($C_{11}H_{18}O_7S$) C, H, S.

Ammonium 2,6-Anhydro-3,8-dideoxy-8-thio-D-*glycero*-D-*talo*-octonate (17). Compound **16** (65 mg, 0.22 mmol) was deacetylated with sodium methoxide (1–2 equiv) in MeOH under dry and oxygen-free conditions for 20 min. Neutralization with Amberlite IR 120 (H^+) was followed by filtration and concentration. Chromatography on silica gel with EtOAc–toluene–MeOH 8:1:1 gave the methyl ester of **17** (35 mg, 67%): R_f 0.38 (EtOAc–toluene–MeOH 8:1:1); ^{13}C NMR (CD_3OD) δ 173.9 (C-1), 77.4, 74.1, 70.9, 68.4, 68.2 (C-2,4,5,6,7), 52.8 (OCH_3), 29.8 (C-3), 29.8 (C-8). The methyl ester was hydrolyzed in 0.5 M NaOH (0.8 mL) for 30 min under N_2. Neutralization with Amberlite IR 120 (H^+) and filtration followed by addition of NH_4OH to pH 8 and concentration gave **17** (33 mg, 94%): R_f 0.5 ($CHCl_3$–MeOH–H_2O 5:1:1); ^{13}C NMR (D_2O) δ 178.9 (C-1), 75.6, 75.1, 70.2, 67.2, 67.2 (C-2,4,5,6,7), 29.4, 29.0 (C-3,8); ^1H NMR (D_2O) δ 4.32 (d, 1 H, *J* = 6.5 Hz, H-2), 3.96 (br s, 1 H, H-5), 3.9–3.65 (m, 2 H, H-4,6), 3.62 (d, 1 H, *J* = 9 Hz, H-6), 2.9–2.7 (m, 2 H, H-8), 2.21 (dd, 1 H, *J* = 12 Hz, 5 Hz, H-3*e*), 1.99 (ddd, 1 H, *J* = 13 Hz, 12 Hz, 6.5 Hz, H-3*a*); FAB-MS, $(M - H)^-$ at *m/z* 237.0443 (calcd 237.0433).

Enzymology. A. Preparation of the Enzymes and Lipid A Precursor. 3-Deoxy-D-*manno*-octulosonate cytidylyltransferase (CMP-KDO synthetase; EC 2.7.7.38) and KDO-lipid A transferase were prepared from *Escherichia coli* D21 or a mutant of *Salmonella typhimurium* (SL 1102) essentially as described previously[22] and lipid A precursor was obtained[27] from another mutant of *Salmonella typhimurium* (AG 701i50).

B. Enzyme Assays. CMP-KDO Synthetase (CKS). The crude enzyme preparation was added to a mixture consisting of KDO, $MgCl_2$, CTP, Tris·HCl buffer, pH 9.5 (final concentrations were 2.54, 9.09, 12.73, and 200 mM, respectively), and a solution of the test compound. The total volume was 550 μL. The mixture was incubated at 25 °C, and the reaction was terminated at 0 and 13 min by transferring 125 μL into 300 μL of ice-cold ethanol, followed by addition of 212 μL of 1 M $NaBH_4$. The mixture was left for 10 min at room temperature, then 560 μL of 0.5 M H_2SO_4 was added, and the mixture was kept at room temperature for 5 min before incubation at 37 °C for 10 min. After centrifugation, the amount of KDO in the supernatant, being derived from CMP-KDO, was determined by the thiobarbituric acid method in the following way: 500 μL of the supernatant was mixed with 250 μL of 0.04 M HIO_4 in 0.0625 M H_2SO_4 and left for 10 min at room temperature. Then 250 μL of 3.5% $NaAsO_2$ in 0.5 M HCl was added, and the mixture was allowed to stand until colorless whereupon 500 μL of 0.5% thiobarbituric acid was added. The mixture was heated at 100 °C for 15 min, 1 mL of DMSO was added, and the mixture was cooled to room temperature. The optical density was then read at 548 nm, and the amount of KDO bound in CMP-KDO was calculated from a standard curve.

CMP-KDO Synthetase–KDO-Lipid A Transferase (CKS–KLT). In this combined assay, the incorporation of [1-^{14}C]KDO into a trichloracetic acid insoluble product was determined. The final incubation mixture (150 μL) contained 0.16 mM [1-^{14}C]KDO (2.8 μCi/μmol), 0.5 mM CTP, 0.4% Alfonic 1012-6, lipid A precursor, 110 mM Tris·HCl buffer, pH 8.0, 10.6 mM $MgCl_2$, CMP-KDO synthetase, and KDO-lipid A transferase from the crude enzyme preparations. The enzyme reaction was initiated by adding a mixture of the last four mentioned components to the other components. The incubation was performed at 25 °C and terminated by pipetting 50 μL of the incubation mixture on to a filter paper (Munktell no. 5), which was put into a beaker containing 10% trichloroacetic acid. The filters were washed extensively with 10% trichloroacetic acid and counted in a universal liquid scintillation cocktail (Insta-Gel, Packard) employing a Packard Tri-Carb 460 CD liquid scintillation system. The inhibition (expressed in percent) was calculated by comparing the enzyme activity with and without the test compound, respectively.

CKS–KLT with Preincubation. The preincubation mixture (62 μL) contained 0.39 mM [1-^{14}C]KDO (2.8 μCi/μmol), 1.22 mM CTP, 97.6 mM Tris·HCl buffer, pH 8.0, 9.4 mM $MgCl_2$, and crude CMP-KDO synthetase. After incubation for 15 min at 25 °C, a mixture (88 μL) consisting of 0.71% Alfonic 1012-6, 119 mM Tris·HCl buffer, pH 8.0, 11.4 mM $MgCl_2$, lipid A precursor, and crude KDO-lipid A transferase and the test compound were added.

(27) Rick, P. D.; Fung, L. W.-M.; Ho, Ch.; Osborn, M. J. *J. Biol. Chem.* **1977**, *252*, 4904.

The incubation was continued for another 10 min. The enzyme reaction was terminated and the activity was measured in the same way as for the CKS-KLT assay. If no difference in enzyme activity was observed with or without an active compound, this indicates that the test compound inhibited the CMP-KDO synthetase.

Determination of Minimum Inhibitory Concentration (MIC). Twofold serial dilutions of the test compound were made in Mueller-Hinton broth (Oxoid) in the wells of a Microtitre plate, giving a final volume of 100 μL/well. Bacteria (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. **3**, 106174-63-4; **4**, 116337-35-0; **5**, 116337-38-3; **5** (8-*o*-trityl derivative), 116337-36-1; **5** (8-*o*-tosyl derivative), 116337-43-0; **6**, 116337-39-4; **6** (8-*o*-trityl derivative), 116337-37-2; **7**, 116337-29-2; **8**, 116337-30-5; **9**, 116337-24-7; **10**, 116337-31-6; **10** [8-(de-iodo) derivative], 116337-32-7; **11**, 107584-46-3; **11** (methyl ester), 116337-33-8; **12**, 116337-41-8; **13**, 106174-48-5; **14**, 106174-79-2; **15**, 107584-44-1; **16**, 116337-34-9; **17**, 116363-83-9; **17** (methyl ester), 116337-47-4; EC 2.7.7.38, 37278-28-7.

Synthesis, DNA Binding, and Biological Evaluation of Synthetic Precursors and Novel Analogues of Netropsin[1]

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A series of oligopeptides have been synthesized that are structurally related to the natural agent netropsin. The binding constants to double-stranded polynucleotides as well as the cytostatic activity against both murine human tumor cell lines and the in vitro activity against a range of DNA and RNA viruses have been determined for these novel compounds and some of their synthetic precursors. 1-Methyl-5-nitropyrrole-2-carboxylic acid methyl ester (**4**), *N*-[[1-methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrol-2-yl]carbonyl]-L-alanine *tert*-butyl ester (**28**), and *N*-[[1-methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrol-2-yl]carbonyl]-L-alanyl-L-alanine *tert*-butyl ester (**29**) showed modest inhibitory effect on tumor cell proliferation (CD_{50} = 26–85 μg/mL). Of all the compounds that were evaluated, **28** proved the most potent antiviral agent. It was inhibitory to parainfluenza-3 virus and Coxsackie virus B4 in Vero cells at a concentration of 20 μg/mL.

Netropsin[2] (synonym congocidin) is a highly basic oligopeptide containing two 4-amino-1-methylpyrrole-2-carboxylate residues that belongs to a family of natural substances including distamycin,[3] anthelvencin,[4] kikumycin,[5] amidomycin,[6] and noformycin[7] (Figure 1).

To date, netropsin has attracted considerable attention because of its noteworthy biological activities and DNA binding capacity.[8] Thus netropsin appeared to be primarily active against Gram-positive and Gram-negative bacteria[9,10] and it was also endowed with antiparasitic properties.[11-13] As an antiviral drug, it was reported to inhibit the multiplication of DNA viruses such as vaccinia virus,[14] herpes simplex virus,[15] swine fever virus,[16] and several retroviruses such as Rous Sarcoma virus,[17] murine leukemogenic Rauscher virus,[18] and feline leukemia virus.[19] Netropsin is also of prime interest in molecular biology owing to its strong minor groove nonintercalative binding to double-stranded B-DNA and its $(A{\cdot}T)_4$ sequence selectivity.[20] Through it has not been conclusively proven, it is generally believed that its DNA binding property is responsible for its biological activity.

Netropsin was first isolated from the fermentation medium of various *Streptomyces* species.[11,12,21] Since the report of its definite structure,[22] several total syntheses of the parent compound[24,29] as well as of dimeric derivatives[30-32] and certain analogues[33-46] of netropsin have been reported. These analogues consisted of compounds in which (i) the number of pyrrole units varied from one to three and/or the aminopropionamidine side chain was replaced by various aminoalkylamidine side chain was replaced by various aminoalkylamidine moieties,[33-36] (ii) the mode of pyrrole ring substitution was changed from

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(2) Julia, M.; Préau-Joseph, N. *Bull. Soc. Chim. Fr.* **1967**, 4348.
(3) Arcamone, F.; Orezzi, P. G.; Barbieri, W.; Nicolella, V.; Penco, S. *Gazz Chim. Ital.* **1967**, *67*, 1097.
(4) Probst, G. W.; Hoehn, M. M.; Woods, B. L. *Antimicrob. Agents Chemother.* **1965**, 789.
(5) Kikuchi, M.; Kumagai, K.; Ishida, N.; Ito, Y.; Yamaguchi, T.; Furumai, T.; Okuda, T. *J. Antibiot., Ser. A* **1965**, *18*, 243.
(6) Nakamura, S.; Karasawa, K.; Yonehara, H.; Tanaka, N.; Umezawa, H. *J. Antibiot., Ser. A* **1961**, *14*, 103.
(7) Diana, G. D. *J. Med. Chem.* **1973**, *16*, 857.
(8) (a) Hahn, F. E. In *Antibiotics III. Mechanism of Action of Antimicrobial and Antitumor Agents*: Corcoran, J. W., Hahn, F. E., Eds.; Springer-Verlag: New York, 1975; pp 79–100. (b) Zimmer, C. In *Progress in Nucleic Acid Research and Molecular Biology*; Cohn, W. E., Ed.; Academic Press: New York, 1975; Vol. 15, pp 285–318.
(9) Thrum, H.; Haupt, I.; Bradler, G.; Zimmer, C.; Reinert, K. G. In *Antimicrobial and Antineoplastic Chemotherapy*; Czech. Med. Press: Prague, 1972; Vol. I, Part 2, pp 819–822.
(10) Schuhmann, E.; Hapt, I.; Thrum, H.; Tabubeneck, U. *Z. Allg. Microbiol.* **1974**, *14*, 321.
(11) Cosar, C. R.; Ninet, L.; Pinnert-Sindico, S.; Preudhomme, J. *C. R. Hebd. Seances Acad. Sci.* **1952**, *234*, 1498.
(12) Depois, C.; Ninet, L. *Abstr. Int. Congr. Microbiol. (Rome) 6th* **1953**, *162*, 241.
(13) Nakamura, S.; Yonehara, H.; Umezawa, H. *J. Antibiot., Ser. A* **1964**, *17*, 220.
(14) (a) Schabel, F. M.; Laster, W. R.; Brockman, R. W.; Skipper, H. E. *Proc. Soc. Exp. Biol. Med.* **1953**, *83*, 1. (b) Werner, G. H.; Maral, R. *Actual. Pharmaceut. Fr.* **1963**, *21*, 133.
(15) Becker, Y.; Neuman, S.; Levitt-Hadar, J. *Isr. J. Med. Sci.* **1972**, *8*, 1225.

0022-2623/89/1832-1074$01.50/0