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2-Deoxy-2-(substituted-methyl)analogs of β-Kdo*p*

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ABSTRACT

Analogs of 3-deoxy-D-*manno*-octulosonic acid (Kdo) having the β-anomeric hydroxyl group replaced by a substituted-methyl group were prepared from Kdo. The substituted-methyl groups were derived from the carboxyl group of Kdo, whereas the carboxyl group in the analogs was introduced by the stereospecific reaction of the ketose azidomethyl acetate **12d** with Me_3SiCN to give the nitrile **16**. Hydrolysis of the nitrile group of **16** gave the azidocarboxylic acid **17a**. Hydrogenation of **17a** gave the amino acid **4a**. Key steps in the preparation of the 2-hydroxymethyl and 2-phosphonomethyl analogs **4b** and **4c**, respectively, were the selective reduction of the azidomethyl derivative **17a** to the aminomethyl derivative **17b**, conversion of **17b** by deamination to the β-lactone **19**, and nucleophilic opening of the β-lactone ring of **19** with hydroxide and triethyl phosphite, respectively, to give **4b** and **4c**. An interesting contrast in the reaction with azide of the methyl and the (methylthio)methyl glycosides of the 1-*O*-alkylsulfonates **9b** and **9f**, respectively, was observed. Whereas the former reacted by direct displacement to give the unrearranged azide **9d**, the reaction of the latter occurred with sulfur participation to give the rearranged azide **11**.

INTRODUCTION

It was recently established in these laboratories[1] that, of the four interconverting tautomers of Kdo[2], only the β-pyranose **1** is bound to CMP-Kdo synthetase. Consistent with this observation was the discovery here[3] that the 2,6-anhydro-3-deoxyaldonic acid analog **2** is a potent inhibitor of CMP-Kdo synthetase, whereas its 2-epimer **3** is virtually inactive. Although the analog **2** is locked to simulate the favorable β-pyranose form, it lacks the potential hydrogen-bond donor or acceptor present at C-2 in the β-pyranose tautomer (**1**) of Kdo. In the hope of preparing more potent inhibitors of CMP-Kdo synthetase based on the structure of **2**, we planned the syntheses of 2-deoxy-2-(substituted-methyl) analogs **4a**, **4b** and **4c** of β-Kdo*p* (**1**). It was hoped that the substituted methyl groups of these analogs, with their potential hydrogen-bond donor or acceptor properties might interact with a binding site on CMP-Kdo synthetase normally occupied by HO-2 of **1**, leading to stronger binding and consequently better competitive inhibitors. Our approach to the syntheses of **4a**, **4b** and **4c** involved utilization of the Kdo carboxyl group as the precursor of the 2-(substituted-methyl) group. Subsequent application of the reaction of Me_3SiCN at the anomeric center of a suitable ketose derivative **5**, thus derived, would introduce into the product **6** a nitrile group in a manner analogous to the reactions of aldose glycosyl acetates[4]. It was hoped that the favored stereochemistry of the reaction would lead to the glycosyl cyanide **6**, which would be the

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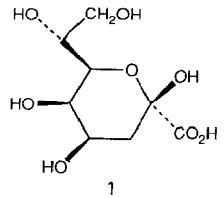

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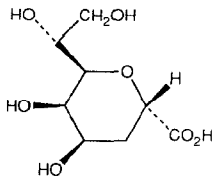

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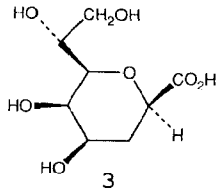

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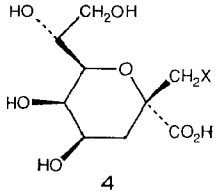

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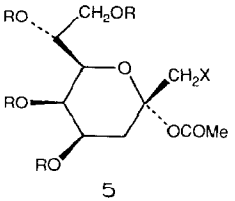
b X = OH
c X = PO_3H_2



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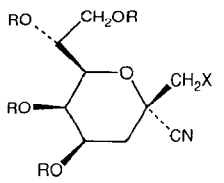

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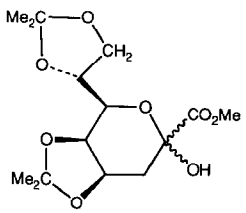
precursor of the carboxylic acids **4a**, **4b** and **4c**. Alternative syntheses of C-glycosyl derivatives of Kdo utilizing enolate chemistry have been reported[5].

DISCUSSION

Treatment of Kdo with 2,2-dimethoxypropane in methanol, catalyzed by acidic resin gave the diisopropylidene acetal[3] **7**. The latter was converted by *O*-methylation into the methyl glycoside **8a**. Sodium borohydride reduction of **8a** gave the alcohol **9a** which was converted into the azide **9d** with sodium azide in DMF either via the methanesulfonate **9b** or the trifluoromethanesulfonate **9c**. Whereas the yield of the methyl glycoside **8a** by *O*-methylation of **7** was low, it was found that Albright–Goldman conditions[6] applied to **7** gave the (methylthio)methyl glycoside **8b** in good yield. It was, therefore, hoped that the 2-*O*-(methylthio)methyl group of **8b** would provide a suitable protecting group for the C-2 of **7** required for preparation of the 2-(substituted-methyl) analogs. Sodium borohydride reduction of **8b** gave the desired alcohol **9e** in good yield, and the latter was readily converted into the methanesulfonate **9f**. In contrast to the vigorous conditions (125°, three days) required for conversion of the methanesulfonate **9b** of the methyl glycoside into the azide **9d** with sodium azide in



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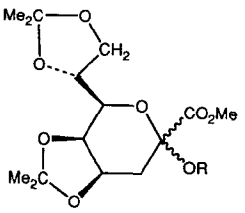

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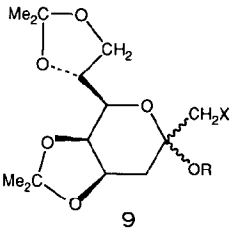
a R = Me
b R = CH_2SMe



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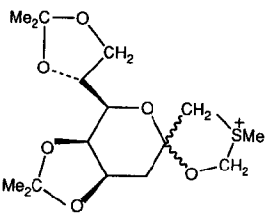
a X = OH, R = Me
b X = OSO_2Me, R = Me
c X = OSO_2CF_3, R = Me
d X = N_3, R = Me
e X = OH, R = CH_2SMe
f X = OSO_2Me, R = CH_2SMe
g X = N_3, R = CH_2SMe



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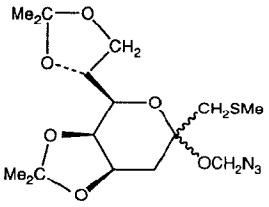
DMF, displacement of the methanesulfonate group of the (methylthio)methyl ether **9f** occurred under relatively mild conditions (100°, 20 h), under which the methanesulfonate **9b** of the methyl glycoside was quantitatively recovered. The difference in the ease of reaction with sodium azide in DMF of **9b** and **9f** was accounted for by the discovery that reaction of **9f** occurred with sulfur participation of the 2-*O*-(methylthio)methyl group *via* the five-membered cyclic sulfonium ion **10**. The latter **10** on reaction with azide ion gave rise to the rearranged product **11** rather than the product of direct displacement **9g**.

Confirmation of the structure of **11** was obtained from the nature of the products formed from **11** by mild acid-catalyzed hydrolysis. Whereas treatment of the methyl glycoside **9d** with 3:2 acetic acid–water selectively removed the isopropylidene groups to give the deprotected methyl glycoside **12a**, the same treatment of **11** gave a mixture of two products, both of which had lost not only the isopropylidene groups, but also the labile *O*-azidomethyl group. The more-polar product was characterized as one of the two possible pyranose anomers **13**, of the (methylthio)methyl ketoside by the magnitude of its coupling constant $J_{3,4}$ (12.6 Hz), which is characteristic of vicinal diaxial protons in six-membered rings. The ^{13}C-n.m.r. spectrum of this product lacked a resonance near 86 p.p.m., which is characteristic of the C-5 carbon atoms of the furanose forms[3a] of Kdo. ^{13}C-N.m.r. studies showed that **13** was almost completely



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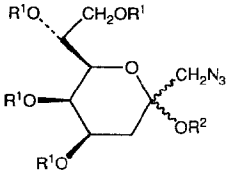
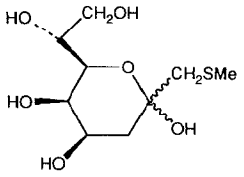


12

a $R^1 = H$, $R^2 = Me$
b $R^1 = Bn$, $R^2 = Me$
c $R^1 = Bn$, $R^2 = H$
d $R^1 = Bn$, $R^2 = COMe$



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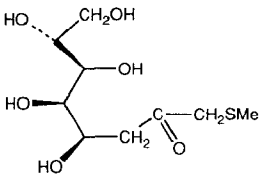


13a

converted into the less-polar product in acetic acid–water solution in about 20 h. ^{1}H-N.m.r. studies with **13** showed exchange in D_2O of the methylene protons of the (methylthio)methyl group as well as the C-3 methylene protons which may occur via the open form **13a**.

The less-polar product **14** was characterized as a furanose structure by the resonance of its C-5 carbon at 86 p.p.m. (Table I), which is close to that observed[2a] for the C-5 carbon resonances of both of the anomers of the furanose forms of Kdo. An anhydro form was suggested by the high-resolution f.a.b. mass spectrum. This was confirmed by n.m.r. studies (Table I), which established the formation of a C-2 to C-7 bridge from the absence of the hydroxyl groups at C-2 and C-7. The location of the hydroxyl groups in **14** was established both by the deuterium-induced β-isotope effects on the ^{13}C resonances only on their hydroxyl-bearing carbons at C-4, C-6, and C-8, and by the vicinal coupling constants in Me_2SO between the C-4, C-6, and C-7 protons and the protons attached to their respective hydroxyl groups (Table I). Reduction of the azidomethyl glycoside **11** by $LiAlH_4$ gave rise to a product the n.m.r. spectrum and analysis of which were compatible with the ring-opened methylthio diol **15**.

The azidomethyl methyl glycoside **12a** was converted into the tetra-*O*-benzyl derivative **12b**. Acid-catalyzed hydrolysis of **12b** to give **12c**, followed by acetylation, gave the glycosyl acetate **12d**. Conversion of the glycosyl acetate **12d** to the nitrile **16** was effected with Me_3SiCN in acetonitrile, catalyzed by boron trifluoride etherate. Base-catalyzed hydrolysis of **16** gave the carboxylic acid **17a**. The conformation of the pyranose ring of **17a** was established as **17a′** from the coupling constant $J_{3a,4a} = 12.3$ Hz.

TABLE I[a]

N.m.r. Data of Compound **14**

^{1}H-N.m.r. data (Me_2SO-d_6) δp.p.m. vs. δ_{Me_2SO} = 2.5		*Coupling constants (Me_2SO) (Hz)*	*^{1}H-N.m.r. data (D_2O) δp.p.m. vs. δ_{HOD} = 4.8*	*^{13}C-N.m.r. data (D_2O) δp.p.m. vs. δ_{CD_3CN} = 1*		*Δδ (H_2O/D_2O)*
SMe	2.12		2.22	SMe	17.3	[b]
H-1	2.74(2H)		2.95, 3.01	C-1	40.0	[b]
H-2				C-2	109.3	−0.057
H-3*a*	1.91		2.08	C-3	43.8	0.043
H-3*b*	2.32	$J_{3a,3b}$ 11.7	2.64	C-4	69.9	0.072
H-4	4.40	$J_{3a,4}$ 2.0, $J_{3b,4}$ 6.0	4.63	C-5	86.0	0.015
HO-4	4.95	$J_{OH,4}$ 3.7		C-6	63.0	0.071
H-5	3.96	$J_{4,5}$ [b]	4.27	C-7	76.2	0.020
H-6	3.37	$J_{5,6}$ 3.8	3.69	C-8	62.0	0.082
HO-6	5.20	$J_{OH,6}$ 3.9				
H-7	3.37	$J_{6,7}$ 4.8	3.50			
H-8*a*	3.39	$J_{7,8a}$ 4.5	3.68			
H-8*b*	3.56	$J_{7,8b}$ 1.8, $J_{8a,8b}$ 10.0	3.79			
HO-8	4.49	$J_{OH,(8a,8b)}$ 4.7				

[a] Proton assignments were determined by selective homonuclear decoupling. Carbon ^{13}C assignments were determined by a 2-D proton–carbon correlation. The ^{13}C deuterium-induced isotope shifts were determined by obtaining a ^{13}C-n.m.r. spectrum in both H_2O and D_2O solutions. The resonances of carbons bearing hydroxyl groups are shifted upfield when the OH proton is substituted with a deuteron. As the β-isotope shift is larger than other long-range isotope shifts, the carbons bearing hydroxyl groups are easily identified as C-4, C-6, and C-8. This is consistent with proton data obtained in Me_2SO. [b] Very small.

The configuration of **17a** at C-2 could thus be established by heteronuclear decoupling by the method of Unger[7], since the coupling constant $J_{3a,H\text{-}1}$ = 8.1 Hz established the antiperiplanar relationship between the carboxyl group and the C-3-axial proton. This method was used to establish the C-2 configurations of related *C*-glycosyl analogs of Kdo.[5]

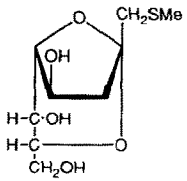
Catalytic hydrogenation of **17a** gave the amino acid **4a**.

Treatment of the azido acid **17a** with Zn in acetic acid gave a mixture of the amino acid **17b** (49%) and the β-lactone **19** (40%). Deamination of the amino acid **17b** with sodium nitrite in aqueous acetic acid gave the β-lactone **19**.

Formation of the β-lactone by nitrous acid deamination of **17b**, which must proceed *via* the diazonium ion **20** (Scheme 1), suggests that partitioning of the azido acid **17a** between the amino acid **17b** and the lactone **19** on treatment with Zn and acetic acid occurs *via* the isomeric azoamino compounds **21** and **22** (Scheme 1). The former leads to the lactone **19** *via* the diazonium ion **20**, while the latter leads to the amino acid **17b**.

Base-catalysed hydrolysis of the lactone **19** smoothly gave rise to the hydroxy acid **17c** which was *O*-debenzylated by catalytic hydrogenation to give **4b**.

Opening of the lactone ring of **19** with triethyl phosphite gave the triethyl ester **23**, which was deprotected by base-catalysed hydrolysis and catalytic hydrogenation to give



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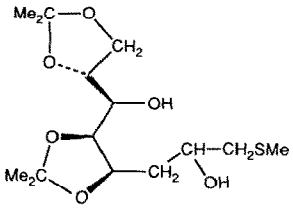

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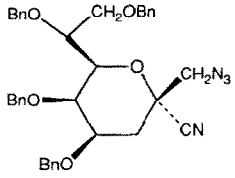

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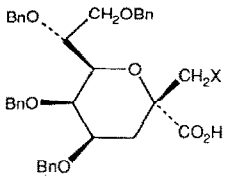
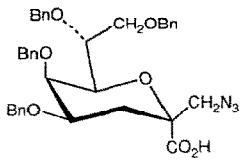
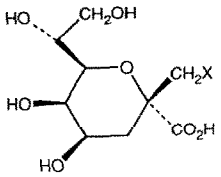


17

a X = N_3
b X = NH_2
c X = OH



17a



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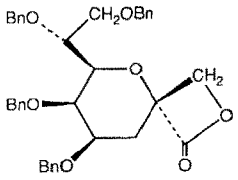

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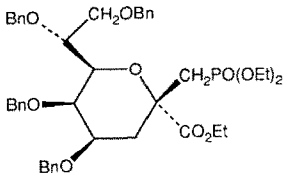

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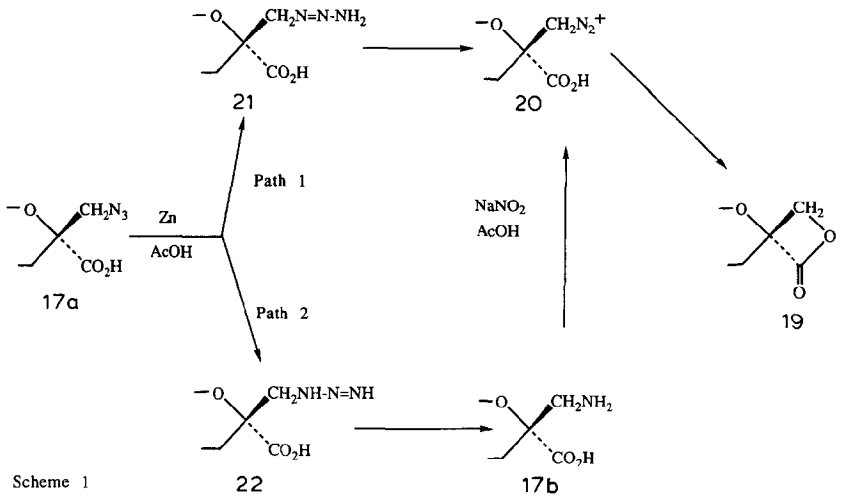

Scheme 1

the phosphonic acid **4c**. The corresponding reaction of *β*-propiolactone has been reported[8].

All of the new analogs **4a**, **4b** and **4c** showed only weak activities as CMP-Kdo synthetase inhibitors compared with 2,6-anhydro-3-deoxy-D-*glycero*-D-*galacto*-octonic acid ("2-deoxy-*α*-carboxy Kdo", **2**). The concentrations [I_{50} (μM] of compounds **2**, **4a**, **4b**, and **4c** which caused 50% inhibition of CMP-Kdo synthetase were 10.5, 11 000, 1100, and 9200, respectively.

EXPERIMENTAL

General methods. — N.m.r. spectra were determined at 300 MHz with a General Electric GN 300 nuclear magnetic resonance spectrometer, high-resolution mass spectra with a Kratos MS50 mass spectrometer, and i.r. spectra with a Perkin–Elmer model 283B or a Nicolet 60 SX FT spectrometer. All compounds had i.r. absorptions characteristic of the chromophores present. Optical rotations were determined with a Perkin–Elmer model 241 digital polarimeter. Gravity chromatography was performed with Merck, Darmstadt 70–230 mesh silica gel, and flash chromatography was carried out with Merck, Darmstadt 230–400 mesh silica gel. Extractions with $CHCl_3$ were carried out by shaking the mixtures or solutions with mixtures of $CHCl_3$ and 5% aq. $NaHCO_3$. The $CHCl_3$ solutions were separated and dried ($MgSO_4$), the $CHCl_3$ was evaporated under diminished pressure, and the residues were dried under high vacuum.

Methyl 3-deoxy-4,5;7,8-di-O-*isopropylidene*-D-manno-*2-octulopyranosonate* (**7**). — A mixture of 10 g (36.6 mmol) of Kdo ammonium salt, 2,2-dimethoxypropane (200 mL), dry MeOH (200 mL), and MeOH-washed and dried AG 50Wx8 (H^+) resin (20 g) was stirred for 3 d at room temperature. The mixture was filtered, the filtrate was evaporated and the residue flash chromatographed (80:20:1.5:1 PhMe–EtOAc–Me-

OH–Et_3N) to yield 6.4 g (53%) of **7** as a white solid. An analytical sample was prepared by gravity chromatography with the same solvent system; m.p. 132–134°, $[\alpha]_D^{20}$ +28.2° (*c* 1, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 1.92 (dd, 1 H, $J_{3,4}$ 4.9, $J_{3,3'}$ 14.4 Hz, H-3), 2.51 (dd, 1 H, $J_{3',4}$ 6.6 Hz, H-3'), and 3.82 (s, 3 H, CO_2Me).

Anal. Calc. for $C_{15}H_{24}O_8$: C, 54.20; H, 7.28. Found: C, 53.57; H, 7.44.

Methyl (methyl 3-deoxy-4,5;7,8-di-O-*isopropylidene*-D-manno-*2-octulopyranosid)onate* (**8a**). — Methyl iodide (1.87 mL, 30 mmol) was added to a stirred mixture of 3.32 g (10 mmol) of **7**, 4.63 g (20 mmol) of Ag_2O, and 0.11 g (0.3 mmol) of Bu_4NI in 80 mL of dry DMF. After stirring for 24 h, the mixture was filtered and the insoluble salts washed with DMF. The combined filtrates were evaporated under diminished pressure. Chloroform was added to the residue and the solution was filtered through a Celite mat. The filtrate was washed with H_2O, dried ($MgSO_4$) and evaporated. Flash chromatography with 1:10 EtOAc–$CHCl_3$ gave 1.3 g (38%) of **8a** as a solid; m.p. 114–117°; ^{1}H-n.m.r. ($CDCl_3$): δ 1.32, 1.38, 1.43, 1.45 (4 s, 12 H, CMe_2), 1.87 (dd, 1 H, $J_{3,4}$ 3, $J_{3,3'}$ 15 Hz, H-3), 2.78 (dd, 1 H, $J_{3',4}$ 3.6 Hz, H-3'), 3.23 (s, 3 H, OMe), and 3.78 (s, 3 H, CO_2Me).

Anal. Calc. for $C_{16}H_{26}O_8$: C, 55.48; H, 7.57. Found: C, 54.60; H, 7.50.

*Methyl 3-deoxy-4,5;7,8-di-*O-*isopropylidene*-D-manno-*2-octulopyranoside* (**9a**). — To a stirred solution of 776 mg (2.34 mmol) of the ester **8a** in a 18 mL of MeOH, cooled in an ice–water bath, was added a freshly prepared solution of 614 mg (16.2 mmol) of $NaBH_4$ in 6 mL of water. Stirring was continued with cooling for 1 h and then at room temperature overnight. Extraction with $CHCl_3$ gave a syrup which on chromatography using 1:1 EtOAc–$CHCl_3$ gave 527 mg (74%) of **9a** as a syrup; ^{1}H-n.m.r. ($CDCl_3$): δ 1.33, 1.36, 1.42, 1.44 (4s, 12 H, CMe_2), 1.68 (dd, 1 H, $J_{3,4}$ 3.9, $J_{3,3'}$ 15.9 Hz, H-3), 2.47 (dd, 1 H, $J_{3',4}$ 3.6 Hz, H-3'), and 3.26 (s, 3 H, OMe).

*Methyl 1-azido-1,3-dideoxy-4,5;7,8-di-*O-*isopropylidene*-D-manno-*2-octulopyranoside* (**9d**). — To a stirred solution of the alcohol (**9a**) [prepared by $NaBH_4$ reduction of 1.38 g (3.98 mmol) of ester **8a**, as just described] in 22 mL of pyridine, cooled in an ice–water bath, was added 1.10 mL (6.53 mmol) of $(CF_3SO_2)_2O$. Stirring was continued with cooling for 1 h. Extraction with $CHCl_3$ gave the trifluoromethanesulfonate **9c** as a syrup. The latter was immediately dissolved in 34 mL of Me_2SO and treated with 1.05 g (16.2 mmol) of NaN_3. The resulting solution was heated for 3 h at 75°. Extraction with $CHCl_3$ gave a syrup which on chromatography with 10:1 $PhMe_3$–EtOAc gave 0.685 g (50%) of **9d**; $[\alpha]_D^{25}$ +41° (*c* 1, MeOH); ^{1}H-n.m.r. ($CDCl_3$): δ 1.33, 1.36, 1.41, 1.43 (4s, 12 H, CMe_2), 1.66 (ddd, 1 H, $J_{3,3'}$ 16.5, $J_{3,4}$ 3.9 Hz, H-3), 2.41 (dd, 1 H, H-3'), 3.27 (s, 3 H, OMe), 3.38 (d, 1 H, J_{AB} 13.5 Hz, $CH_AH_BN_3$), and 3.65 (dd, 1 H, $J_{B,H\text{-}3}$ 1.5 Hz, $CH_AH_BN_3$).

Anal. Calc. for $C_{15}H_{25}N_3O_6$: C, 52.46; H, 7.34; N, 12.24. Found: C, 52.78; H, 7.56; N, 12.19.

*Methyl 3-deoxy-4,5;7,8-di-*O-*isopropylidene-1-*O-*methylsulfonyl*-D-manno-*2-octulopyranoside* (**9b**). — To a stirred solution of 514 mg (1.6 mmol) of the alcohol **9a** in 7 mL of pyridine, cooled in an ice–water bath was added 0.30 mL (3.8 mmol) of $MeSO_2Cl$. Stirring was continued with cooling for 1 h and then for 1.5 h at room temperature. Extraction with $CHCl_3$ gave the methanesulfonate **9b** as a syrup; ^{1}H-n.m.r. ($CDCl_3$): δ 1.34, 1.38, 1.42, 1.44 (4s, 12 H, CMe_2), 1.66 (ddd, 1 H, $J_{3,4}$ 3.6, $J_{3,3'}$ 16.5 $J_{1,3}$ 1.5 Hz, H-3), 2.46 (dd, 1 H, $J_{3',4}$ 3.6 Hz, H-3'), 3.08 (s, 3 H, OSO_2Me), and 3.32 (s, 3 H, OMe).

Attempted reaction of the methanesulfonate **9b** *with* NaN_3 *in DMF at 100°.* — A. A stirred solution of the methanesulfonate **9b**, prepared as just described, 427 mg (6.57 mmol) of NaN_3, and 15 mL of Me_2SO was heated for 18 h at 100°. Extraction with $CHCl_3$ gave 629 mg of recovered **9b**.

B. A stirred solution of 581 mg (1.46 mmol) of the methanesulfonate **9b**, 849 mg (13 mmol) of NaN_3, and 15 mL of Me_2SO was heated at 125° for 3 d. Extraction with $CHCl_3$ gave 439 mg of syrup which on chromatography using 20:1 $CHCl_3$–EtOAc gave 277 mg (55%) of **9d**, identical with that prepared as already described.

*Methyl [(methylthio)methyl 3-deoxy-4,5;7,8-di-*O*-isopropylidene-*D-manno*-2-octulopyranosid]onate* (**8b**). — Dry Me_2SO (47 mL) was added to a stirred solution of 5.4 g (16 mmol) of **7** in 47 mL of Ac_2O. The solution was stirred for 22 h at room temperature. To the solution was added 500 mL of EtOAc and the resulting solution was washed thoroughly with H_2O, 10% aqueous NaCl, and was evaporated under diminished pressure. The residue was flash chromatographed (1:8 EtOAc–PhMe) to give 5.8 g (91% **8b** as a syrup; ^{1}H-n.m.r. ($CDCl_3$): δ 1.32, 1.38, 1.39, 1.43 (4s, 12 H, CMe_2), 1.88 (dd, 1 H, $J_{3,4}$ 2.7, $J_{3,3'}$ 15.5 Hz, H-3), 2.92 (dd, 1 H, $J_{3',4}$ 3.8 Hz, H-3'), 2.17 (s, 3 H, SMe), and 3.77 (s, 3 H, CO_2Me).

*(Methylthio)methyl 3-deoxy-4,5;7,8-di-*O*-isopropylidene-*D-manno*-2-octulopyranoside* (**9e**). — To a stirred solution of 3.4 g (8.7 mmol) of the methyl ester **8b** in 60 mL of MeOH was added a freshly prepared solution of 2.74 g (72.4 mmol) of $NaBH_4$ in 30 mL of water, and stirring was continued overnight. Extraction with $CHCl_3$ gave the alcohol **9e** as a syrup; ^{1}H-n.m.r. ($CDCl_3$): δ 1.33, 1.37, 1.40, 1.41 (4s, 12 H, CMe_2), 1.58 (dd, 1 H, $J_{3,4}$ 3.6, $J_{3,3'}$ 16.5 Hz, H-3), 2.61 (dd, 1 H, $J_{3',4}$ 3.0 Hz, H-3'), 2.28 (s, 3 H, SMe), 2.96 (t, 1 H, $J_{1,OH}$ 7.2 Hz, disappears on D_2O exchange, OH), 3.67 (d, 2 H, singlet after D_2O exchange H-1), 4.56 (d, 1 H, J_{AB} = 12 Hz, H_A of OCH_AH_BSMe), and 4.85 (d, 1 H, H_B).

*(Methylthio)methyl 3-deoxy-4,5;7,8-di-*O*-isopropylidene-1-*O*-methylsulfonyl-*D-manno*-2-octulopyranoside* (**9f**). — To a stirred solution of the alcohol **9e**, prepared as just described, in 45 mL of pyridine, cooled in an ice bath, was added 1.8 mL (23.3 mmol) of $MeSO_2Cl$. Stirring was continued for 1 h with cooling and then for 1 h at room temperature. Extraction with $CHCl_3$ gave the methanesulfonate **9f** as a syrup; ^{1}H-n.m.r. ($CDCl_3$): δ 1.32, 1.38, 1.42, 1.44 (4s, 12 H, CMe_2), 1.87 (dd, 1 H, $J_{3,4}$ 3.0, $J_{3,3'}$ 16.2 Hz, H-3), 2.78 (dd, 1 H, $J_{3',4}$ 4.2 Hz, H-3'), 2.23 (s, 3 H, SMe), and 3.78 (s, 3 H, OSO_2Me).

*Azidomethyl 3-deoxy-4,5;7,8-*O*-di-isopropylidene-1-*S*-methyl-1-thio-*D-manno*-2-octulopyranoside* (**11**). — A stirred solution of the methanesulfonate **9f**, prepared as just described, 2.4 g (36.9 mmol) of NaN_3 and 84 mL of Me_2SO was heated for 20 h at 100°. Extraction with $CHCl_3$ gave 3.68 g of **11** (100% based on **8b**) as a syrup; ^{1}H-n.m.r. ($CDCl_3$): δ 1.31, 1.35, 1.40, 1.43 (4s, 12 H, CMe_2), 1.77 (ddd, 1 H, $J_{3,4}$ 3.6, $J_{3,3'}$ 16.5, $J_{1,3}$ 1.5 Hz, H-3), 2.70 (dd, 1 H, $J_{3',4}$ 4.2 Hz, H-3'), 2.18 (s, 3 H, SMe, 2.86 (d, 1 H, J_{AB} 13.8 Hz, H_A of CH_AH_BSMe), 3.02 (bd, 1 H, H_B), 4.73 (d, 1 H, $J_{A'B'}$ 9.0 Hz, $H_{A'}$ of $OCH_{A'}H_{B'}N_3$), and 4.77 (d, 1 H, $H_{B'}$).

*Methyl 1-azido-1,3-dideoxy-*D*-manno-2-octulopyranoside* (**12a**). — A solution of 277 mg (0.8 mmol) of the azide **9d** in 15 mL of 3:2 AcOH–water was kept for 19 h at

room temperature. Solvent was evaporated under diminished pressure. The residue was dissolved in 10 mL of water and the water was removed by lyophilization leaving 216 mg (100%) of **12a**; ^{1}H-n.m.r. (D_2O): δ 1.81 (t, 1 H, $J_{3a,4} = J_{3a,3e} = 13.2$ Hz, H-3a), 1.97 (dd, 1 H, $J_{3e,4}$ 5.4 Hz, H-3*e*), 3.27 (s, 3 H, OMe), 3.31 (d, 1 H, J_{AB} 13.5 Hz, CH_AH$_B$N$_3$), and 3.66 (d, 1 H, CH$_A$$H_BN_3$).

*3-Deoxy-1-*S*-methyl-1-thio-*D-manno-*2-octulopyranose* (**13**) *and 2,7-anhydro-3-deoxy-1-*S*-methyl-1-thio-α-*D-manno-*2-octulofuranose* (**14**). — A solution of 457 mg (1.2 mmol) of the azide **11** in 25 mL of 3:2 AcOH–water was kept at room temperature overnight. The solvent was evaporated under diminished pressure. The residue was dissolved in water and the water was removed by lyophilization. Chromatography using 10:1 EtOAc–MeOH gave 143 mg (52%) of **14** in the early fractions; $[\alpha]_D^{19}$ +38.4° (*c* 1, MeOH); ^{1}H-n.m.r. (see Table I); exact mass: calc. for $C_9H_{17}O_5S$ (MH)$^+$ 237.0797, found 237.0797; and 62.4 mg (22.5%) of **13** in the later fractions; $[\alpha]_D^{21}$ +43.5° (*c* 1, MeOH); ^{1}H-n.m.r. (D_2O): δ 1.82 (t, 1 H, $J_{3a,3e}$, $J_{3a,4}$ 12.6 Hz, H-3*a*), 1.97 (dd, 1 H, $J_{3e,4}$ 5.4, $J_{3a,3e}$ 12.6 Hz, H-3*e*), 2.18 (s, 3 H, SMe), 2.86 (d, 1 H, J_{AB} 14.1 Hz, CH_AH$_B$SMe), and 2.76 (d, 1 H, CH$_A$$H_B$SMe); exact mass calc. for $C_9H_{19}O_6S$ (MH)$^+$ 255.0902, found 255.0904.

*3-Deoxy-4,5;7,8-di-*O*-isopropylidene-1-*S*-methyl-1-thio-*D-glycero-D-galacto-(or D-talo)-*octitol* (**15**). — To a stirred slurry of 73.5 mg (1.94 mmol) of $LiAlH_4$ in 6 mL of ether was added dropwise a solution of 305 mg (0.78 mmol) of the azide **11** in 10 mL of ether. Stirring was continued for 5 h followed by addition of 3 mL of MeOH. Extraction with ether gave 195 mg of syrup which on chromatography with 1:1 $CHCl_3$–EtOAc gave 138 mg (52%) of **15** as a syrup; ^{1}H-n.m.r. ($CDCl_3$): δ 1.34, 1.38, 1.39, 1.52 (4s, 12 H, CMe_2), and 2.16 (s, 3 H, SMe).

Anal. Calc. for $C_{15}H_{27}O_6S$: C, 53.71; H, 8.11; S, 9.56. Found: C, 53.84; H, 8.32; S, 9.11.

*Methyl 1-azido-4,5,7,8-tetra-*O*-benzyl-1,3-dideoxy-*D-manno-*2-octulopyranoside* (**12b**). — To a stirred solution of 1.19 g (4.52 mmol) of the azide **12a** in 79 mL of DMF, under nitrogen, was added 1.78 g (44.3 mmol) of 60% NaH. Stirring was continued for 0.5 h. The resulting stirred mixture was cooled in an ice–water bath and 0.143 g (0.387 mmol) of Bu_4NI was added followed by 4.3 mL of $PhCH_2Br$. After the addition was complete, stirring was continued for 1 h at room temperature and then overnight at room temperature. Extraction with $CHCl_3$ gave 5.2 g of a dark-brown syrup. Flash chromatography using 50:1 PhMe–EtOAc followed by gravity chromatography using 50:1 PhMe–EtOAc gave 1.49 g (52.8%) of **12b**; $[\alpha]_D^{25}$ +18.7° (*c* 1, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 2.08 (dd, 1 H, $J_{3a,3e}$ 12, $J_{3e,4}$ 5 Hz, H-3*e*), 2.18 (t, 1 H, $J_{3a,4}$ 12 Hz, H-3*a*), 3.18 (s, 3 H, OMe), 3.20 (d, 1 H, J_{AB} 13.5 Hz, CH_AH$_B$N$_3$), 3.35 (d, 1 H, CH$_A$$H_BN_3$).

Anal. Calc. for $C_{37}H_{41}N_3O_6$: C, 71.25; H, 6.63; N, 6.74. Found: C, 71.58; H, 6.80; N, 6.10.

*1-Azido-4,5,7,8-tetra-*O*-benzyl-1,3-dideoxy-*D-manno-*2-octulopyranose* (**12c**). — A stirred solution of 1.49 g (2.39 mmol) of the methyl glycoside **12b** in 70 mL of AcOH was heated to 80° and 25 mL of 2M H_2SO_4 was added over a period of 12 min. After the addition was complete, heating was continued for 1.5 h. The resulting solution was cooled in an ice–water bath and then shaken with a mixture of 200 mL of $CHCl_3$ and 500

mL of ice–water. The $CHCl_3$ solution was separated and washed with 500 mL of 5% $NaHCO_3$ (considerable foaming), and then with 500 mL of water. The aqueous solutions were washed with $CHCl_3$. The $CHCl_3$ solutions were combined and dried ($MgSO_4$). Evaporation of the $CHCl_3$ under diminished pressure and chromatography of the residue with 20:1 $CHCl_3$–EtOAc gave 576 mg (40%) of **12c** as a syrup; ^{1}H-n.m.r. ($CDCl_3$): δ 1.89 (dd, 1 H, $J_{3e,4}$ 6, $J_{3e,3a}$ 12 Hz, H-3*e*), 2.03 (t, 1 H, $J_{3a,4}$ 12 Hz, H-3*a*), 2.93 (d, 1 H, J_{AB} 12.9 Hz, CH_AH$_B$N$_3$), and 3.33 (d, 1 H, CH$_A$$H_BN_3$).

1-O-Acetyl-1-azido-4,5,7,8-tetra-O-benzyl-1,3-dideoxy-D-manno-2-octulopyranose (**12d**). — To a stirred solution of 3.31 g (5.4 mmol) of the azide **12c** in 65 mL of pyridine, cooled in an ice–water bath, was added dropwise over a period of 10 min, 26 mL of Ac_2O followed by 0.1024 g of 4-dimethylaminopyridine. Stirring was continued with cooling for 1 h and then overnight at room temperature. The resulting solution was poured into 500 mL of water and the resulting suspension was cooled in an ice–water bath. Solid $NaHCO_3$ was added portionwise with stirring. Extraction with $CHCl_3$ gave an orange syrup which on chromatography using 20:1 PhMe–EtOAc gave 2.71 g (77%) of **12d**; ^{1}H-n.m.r. ($CDCl_3$): δ 1.92 (s, 3 H, OCOMe), 2.16 (dd, 1 H, $J_{3a,4} = J_{3a,3e} = 12.3$ Hz, H-3*a*), 2.51 (t, 1 H, $J_{3e,4}$ 4.8, $J_{3a,3e}$ 12.3 Hz, H-3*a*), and CH_2N_3 (not resolved).

1-Azido-4,5,7,8-tetra-O-benzyl-1,3-dideoxy-α-D-manno-2-octulopyranosyl cyanide (**16**). — To a stirred solution of 3.85 g (5.9 mmol) of **12d** in 28 mL of dry $MeNO_2$ was added 6.0 mL (45 mmol) of Me_3SiCN, followed by a few drops of BF_3–Et_2O. The solution was stirred at room temperature for 1 h. Extraction with $CHCl_3$ and flash chromatography with 40:1 PhMe–EtOAc yielded 3.45 g (94%) of **16** as a light syrup; $[\alpha]_D^{22}$ +39° (*c* 1, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 2.03 (dd, 1 H, $J_{3e,4}$ 3.3, $J_{3e,3a}$ 12.1 Hz, H-3*e*), 2.28 (t, 1 H, $J_{3a,4}$ 12.1 Hz, H-3*a*), 3.32 (d, 1 H, J_{AB} 12.9 Hz, CH_AH$_B$N$_3$), and 3.50 (d, 1 H, CH$_A$$H_BN_3$).

Anal. Calc. for $C_{37}H_{38}N_4O_5$: C, 71.82; H, 6.19; N, 9.05. Found: C, 71.54; H, 6.03; N, 9.38.

2,6-Anhydro-2-C-(azidomethyl)-4,5,7,8-tetra-O-benzyldeoxy-D-glycero-D-talo-octonic acid (**17a**). — A solution prepared from 3.45 g (5.57 mmol) of **16**, 18 g (0.45 mol) of NaOH, 45 mL of water and 180 mL of EtOH was stirred and heated under nitrogen for 4 days at 70°. The resulting solution was cooled to room temperature and shaken with excess M HCl. Extraction with $CHCl_3$ gave 3.29 g of a syrup which on flash chromatography using 20:2:0.1 PhMe–EtOAc–AcOH gave 2.89 g (81%) of **17a** as a syrup; $[\alpha]_D^{22}$ +10.2° (*c* 1, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 2.15 (t, 1 H, $J_{3a,3e} = J_{3a,4} = 12.3$ Hz, H-3*a*), 2.61 (dd, 1 H, $J_{3e,4}$ 4.2 Hz, H-3*e*), 3.30 (d, 1 H, J_{AB} 12.8 Hz, CH_AH$_B$N$_3$), and 3.49 (d, 1 H, CH$_A$$H_BN_3$).

Anal. Calc. for $C_{37}H_{39}N_3O_7$: C, 69.68; H, 6.16; N, 6.59. Found: C, 69.84; H, 6.84; N, 6.55.

2-C-(Aminomethyl-2,6-anhydro-4,5,7,8-tetra-O-benzyl-3-deoxy-D-glycero-D-talo-octonic acid (**17b**) *and 2,6-anhydro-4,5,7,8-tetra-O-benzyl-3-deoxy-2-C-(hydroxymethyl)-D-glycero-D-talo-octonic acid lactone* (**19**) *by Zn–AcOH reduction of* **17a**. — To a stirred solution of 171 mg (0.27 mmol) of the azido acid **17a** in 8.6 mL of AcOH was added 1.06 g of Zn dust. Stirring was continued for 2 h. The Zn was removed by

filtration. Acetic acid was evaporated under diminished pressure leaving a syrup which on chromatography using 20:2:0.1 $CHCl_3$–MeOH–HCl gave in the early fractions 60 mg (40%) of the β-lactone **19**; $[\alpha]_D^{21}$ +0.47° (*c* 1, MeOH); ^{1}H-n.m.r. ($CDCl_3$): δ 2.26 (dd, 1 H, $J_{3e,4}$ 4.0, $J_{3e,3a}$ 12.6 Hz, H-3*e*), and 2.48 (t, 1 H, $J_{3a,4}$ 12.6 Hz, H-3*a*).

Anal. Calc. for $C_{37}H_{38}O_7$: C, 74.72; H, 6.44. Found: C, 74.46; H, 6.35.

In the later fractions there was obtained 106 mg (49%) of the hydrochloride of the amino acid **17b**; ^{1}H-n.m.r. ($CDCl_3$): broad peaks, no definition.

2,6-Anhydro-4,5,7,8-tetra-O-*benzyl-3-deoxy-2*-C-*(hydroxymethyl)*-D-glycero-D-talo-*octonic acid lactone* (**19**). — A. *By deamination of* **17b**. — To a stirred solution of 102 mg (0.16 mmol) of the hydrochloride of the amino acid **17b**, 2.0 mL of AcOH and 2.0 mL of water was added 82 mg of $NaNO_2$. Stirring was continued for 1 h. Extraction with $CHCl_3$ gave 89 mg of a syrup. Chromatography of 69 mg of the latter using 40:1 PhMe–EtOAc gave 29 mg (31%) of the β-lactone **19**, identical with that prepared as just described.

B. *Preparative.* — To a stirred solution of 1.36 g (2.1 mmol) of the azido acid **17a** in 70 mL of AcOH was added 8.4 g of Zn dust. Stirring was continued for 2 h and the Zn was removed by filtration through a Celite mat. The AcOH was evaporated under diminished pressure leaving a mixture of the amino acid **17b** and the lactone **19**. To a stirred solution of the latter mixture in 40 mL of AcOH and 20 mL of water was added 0.810 g of $NaNO_2$. Stirring was continued for 1 h. The resulting suspension was shaken with a mixture of 500 mL of water and 200 mL of $CHCl_3$. The $CHCl_3$ solution was separated and washed with 500 mL of water. The aqueous solutions were washed in series with four 100-mL portions of $CHCl_3$. The $CHCl_3$ solutions were combined and dried ($MgSO_4$). The solvent was evaporated under diminished pressure and residual AcOH was removed by co-distillation with PhMe under diminished pressure, leaving a syrup which on flash chromatography gave 0.668 g (52.7%) of the β-lactone **19** identical with that prepared as just described.

2,6-Anhydro-4,5,7,8-tetra-O-*benzyl-3-deoxy-2*-C-*(hydroxymethyl)*-β-D-manno-*2*-D-glycero-D-talo-*octonic acid* (**17c**). — A suspension of 458 mg (0.77 mmol) of the lactone **19** in 30 mL of *tert*-BuOH was stirred for 15 min, and 15 mL of 3M NaOH was added. Stirring was continued for 26 h. The resulting suspension was shaken with a mixture of 250 mL of cold 0.5M HCl and 100 mL of $CHCl_3$. The $CHCl_3$ solution was separated and washed with 250 mL of 10% NaCl. The aqueous solutions were washed with $CHCl_3$ and the $CHCl_3$ solutions were combined and dried ($MgSO_4$). Evaporation of solvent left 464 mg of white glass which on flash chromatography with 10:10:0.1 PhMe–EtOAc–AcOH gave 418 mg (89%) of **17c** as a rigid white glass; $[\alpha]_D^{23}$ −2.5° (*c* 1, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 2.11 (t, 1 H, $J_{3a,3e} = J_{3a,4}$ = 12.6 Hz, H-3*a*), and 2.59 (dd, 1 H, $J_{3e,4}$ 4.5 Hz, H-3*e*).

Anal. Calc. for $C_{37}H_{40}O_8$: C, 72.53; H, 6.58. Found: C, 72.34; H, 6.71.

Ethyl 2,6-anhydro-4,5,7,8-tetra-O-*benzyl-2*-C-*(diethylphosphonomethyl)-3-deoxy*-D-glycero-D-talo-*octonate* (**23**). — A stirred solution of 518 mg (0.87 mmol) of **19** in 45 mL of $(EtO)_3P$ was heated for 4 h at 100°. The major portion of the $(EtO)_3P$ was evaporated under high vacuum and residual $(EtO)_3P$ was removed by co-distillation

with PhMe under diminished pressure. Chromatography of the residue using 2:1 PhMe–EtOAc gave 329 mg (50%) of **23** as a syrup; $[\alpha]_D^{20}$ +0.16° (*c* 1, MeOH); ^{1}H-n.m.r. ($CDCl_3$): δ 1.1–1.3 (m, 6 H, OCH_2Me), 2.2–2.4 [m, 3 H, H-3a and $CH_2P(OEt)_2$], and 2.58 (dd, 1 H, $J_{3e,4}$ 5.1, $J_{3e,3a}$ 12 Hz, H-3*e*).

Anal. Calc. for $C_{43}H_{53}O_{10}P$: C, 67.88; H, 6.78. Found: C, 67.26; H, 6.78.

2-C-(Aminomethyl)-2,6-anhydro-3-deoxy-D-glycero-D-talo-*octonic acid hydrochloride* (**4a**). — The azido acid **17a** (393 mg, 0.53 mmol) was catalytically hydrogenated in 100 mL of 0.2M HCl in MeOH with 390 mg of Pd black under 3 atm. of hydrogen. After removal of the major portion of the catalyst by filtration, solvent was evaporated under diminished pressure. Residual HCl was removed by co-distillation with EtOH under diminished pressure. The residue was dissolved in water and filtered through a Millipore type EH membrane. Lyophilization gave 238 mg of a glass. Gel filtration in MeOH through Sephadex LH20 gave 77 mg (100%) of the hydrochloride of **4a** as a glass; $[\alpha]_D^{20}$ +51° (*c* 1, MeOH); ^{1}H-n.m.r. (D_2O): δ 1.72 (t, 1 H, $J_{3a,4} = J_{3a,3e}$ = 12.6 Hz, H-3*a*), 2.30 (dd, 1 H, $J_{3e,4}$ 4.5 Hz, H-3*e*), 3.18 (d, 1 H, J_{AB} 12.6 Hz, $CH_AH_BNH_2$), and 3.25 (d, 1 H, $CH_AH_BNH_2$); exact mass calc. for $C_9H_{18}NO_7$ $(MH)^+$ 252.1083, found 252.1080.

2,6-Anhydro-3-deoxy-2-C-*(hydroxymethyl)*-D-glycero-D-talo-*octonic acid* (**4b**). — The hydroxy acid **17c** (391 mg, 0.64 mmol) was catalytically hydrogenated in 150 mL of 0.2M HCl with 390 mg of Pd black under 3 atm of hydrogen. Catalyst was removed by filtration and solvent was evaporated under diminished pressure. Residual hydrochloric acid was removed by co-distillation with MeOH under diminished pressure. The residue was dissolved in water and filtered through a Millipore type EH membrane. Lyophilization gave (100%) of **4b** as a glass; $[\alpha]_D^{23}$ +58.6° (*c* 1, MeOH); ^{1}H-n.m.r. (D_2O): δ 1.71 (t, 1 H, $J_{3a,3e} = J_{3a,4}$ = 15 Hz, H-3a), and 2.20 (dd, 1 H, $J_{3e,4}$ 4.5 Hz, H-3*e*); exact mass: calc. for $C_9H_{17}O_8$ $(MH)^+$ 253.0923, found 253.0925.

2,6-Anhydro-3-deoxy-2-C-*(phosphonomethyl)*-D-glycero-D-talo-*octonic acid* (**4c**). — A stirred solution of 329 mg (0.43 mmol) **23** in 22 mL of a solution prepared from 2 g of NaOH, 5 mL of water and 20 mL of EtOH was heated overnight at 70° under nitrogen and then allowed to cool. The mixture was shaken with a mixture of 100 mL of $CHCl_3$ and 200 mL of MHCl. The $CHCl_3$ solution was separated and washed with 200 mL of water. The aqueous solutions were washed with $CHCl_3$. The $CHCl_3$ solutions were combined and dried ($MgSO_4$). Evaporation of the $CHCl_3$ left a residue which was hydrogenated with 270 mg of Pd black in 50 mL of 0.2M HCl in MeOH under 3 atm of hydrogen. Conventional isolation of the product gave 164 mg of glass. The latter was dissolved in 10 mL of 2M NaOH and the resulting solution was stirred and heated for 20 h at 70° under N_2. The solution was cooled, diluted with 25 mL of water and acidified by addition of 10 g of Dowex HCR-S (H^+) resin. After removal of the resin by filtration, lyophilization gave 134 mg (98%) of **4c** as a syrup; $[\alpha]_D^{20}$ +46° (*c* 1, MeOH); ^{1}H-n.m.r. (CD_3OD): δ 2.13 (t, 1 H, $J_{3a,3e} = J_{3a,4}$ = 12.6 Hz, H-3*a*), and 2.2–2.4 (m, 3 H, H-3*e* and $CH_2PO_3H_2$); exact mass: calc. for $C_9H_{18}O_{10}P$ $(MH)^+$ 317.0637, found 317.0637.

CMP-Kdo synthetase assay. — CMP-Kdo synthetase was isolated from *Escherichia coli* by the method of Goldman and Kohlbrenner[9]. The reaction was monitored with a coupled assay performed at 30° in semi-micro cuvettes containing 50mM Hepes

(pH 7.6), mM Kdo, 0.5mM $MgCl_2$, mM DTT, 1.8 mg of glycogen, 7.8 units of inorganic pyrophosphatase, 10 units of phosphorylase a, 13 units of phosphoglucomutase, 15 units of D-glucose 6-phosphate dehydrogenase, 0.36 mg of NADP, and CMP-Kdo synthetase in a final volume of 1 mL. After a 6-min. pre-incubation period, the reaction was initiated by the addition of 10 μL of diluted CMP-Kdo synthetase. The change in absorption at 340 nm was measured with a Gilford Response spectrophotometer which was programmed to calculate reaction rates. The reaction was linear between 2 and 5 min after CMP-Kdo synthetase addition. The apparent K_m of Kdo was 0.32mM.

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