A Short Step Synthesis of 3-Deoxy-D-*manno*-2-octulosonic Acid (KDO)

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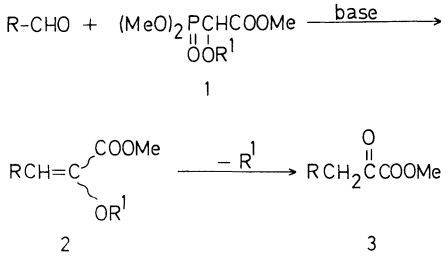
Synopsis. 3-Deoxy-D-*manno*-2-octulosonic acid (KDO) 1,4-lactone was synthesized from *aldehydo*-D-mannose derivatives by Horner–Emmons reaction in two steps.

3-Deoxy-D-*manno*-2-octulosonic acid (2-keto-3-deoxy-octonic acid; KDO) is an integral component of the lipopolysaccharides (LPS) of gram-negative bacteria and connects the lipophilic part of LPS, lipid A, to the core region of polysaccharide through its ketosidic bonds.[1]

Following an identification of the important and unique function of KDO and a determination of its structure, several partial syntheses from simpler sugars could be accomplished. The published methods regarding the synthesis of KDO involve a Wittig reaction of D-mannose derivatives[2] or a reaction of D-arabinose derivatives with di-*t*-butyl oxalacetate.[3] However, these methods required either many steps for the completion of the structure of KDO or a separation of KDO and its isomers.

We now wish to describe a synthesis of KDO involving fewer steps by a Horner–Emmons reaction. The condensation of Horne's phosphonate **1**[4] with aldehydes afforded a carboxymethyl enol ether derivative **2**. We attempted to convert this enol ethers **2** into a keto carboxymethyl derivative **3**, which corresponds to the C-1—C-3 part of KDO.

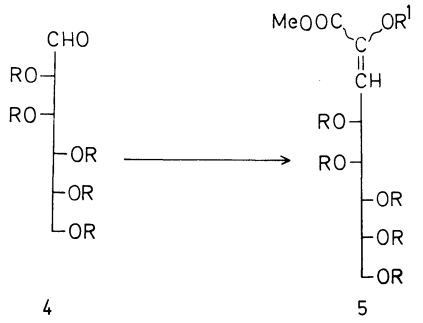


Reactions of acetylated or benzylated *aldehydo*-D-mannose derivatives **4** with **1** using lithium bis(trimethylsilyl)amide as a base in tetrahydrofuran (THF) afforded the expected methyl 3-deoxy-D-*manno*-oct-2-enoate derivatives **5** in reasonable yields; the results are summarized in Table 1. In most cases, the *E*-isomers were formed, predominantly, as judged from the ^{1}H NMR spectra.[6]

In contrast, the reaction of 3,4:5,6-di-*O*-isopropylidene-*aldehydo*-D-mannose, which was not purified after a dethioacetal reaction, with **1** (R^1=–$SiMe_2CMe_3$) did not afford the corresponding **5**, resulting in a complex mixture. 2-*O*-Acetyl-3,4:5,6-di-*O*-isopropylidene-*aldehydo*-D-mannose afforded **5**, though in low yield (25%). When butyllithium or DBU–$LiClO_4$[5] was used as a base, **5** was also obtained, but the yield was lower.

The deprotection step from **5** to KDO 1,4-lactone (**6**) was first investigated by using methyl 4,5,6,7,8-penta-*O*-acetyl-2-*O*-(*t*-butyldimethylsilyl)-3-deoxy-D-*manno*-oct-2-enoate (**5a**). When **5a** was treated with tetrabutylammonium fluoride, the solution immediately became dark brown, indicating a complex mixture. A treatment of **5a** with sodium methoxide in methanol and a subsequent addition of a cation-exchange resin afforded the expected **6**, though in low yield.

Table 1. Yields of 3-Deoxy-D-*manno*-oct-2-enoate Derivatives (**5**)



R	R^1	**5** (%)
-Ac	$-SiMe_2Bu^t$	68 (**5a**)
	-Ac	29 (**5b**)
	$-COOCMe_2CCl_3$	56 (**5c**)
-Bn	$-SiMe_2Bu^t$	70 (**5d**)
	$-COOCMe_2CCl_3$	56 (**5e**)
	-Bn	77 (**5f**)

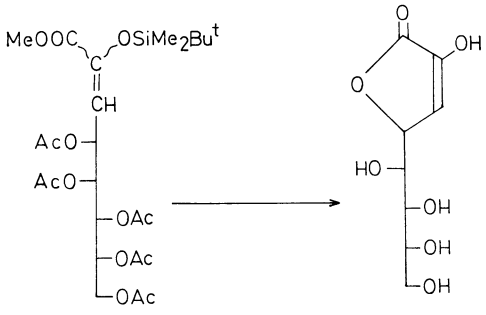
Bn=benzyl.

To improve the yield of **6**, other alkoxides or other methods[7] were investigated. The results are summarized in Table 2. Among these results, the best yield (45%) was obtained with sodium ethoxide in ethanol at −20 °C. The product **6** was readily separated from the reaction mixture by crystallization.

Conversions of other enoates **5b—f** to **6** were also examined, but proved to be unsatisfactory. A trace amount of **6** was obtained from **5c** upon a similar treatment with alkoxide, but not at all from **5b**. Attempts to convert **5d—f** into **6**, involving various methods reported for the removal of benzyl protecting groups (i.e., catalytic hydrogenolysis,[8] reductive cleavage with sodium in ethanol,[9] iodotrimethylsilane,[10] or ethanethiol–boron trifluoride etherate[11]) failed, always resulting in the formation of complex mixtures. When **5d** was first treated with tetrabutylammonium fluoride, the desired 4,5,6,7,8-penta-*O*-benzyl-3-deoxy-D-*manno*-octulosonic acid (66%) was obtained. However, it could not be converted into **6** by catalytic hydrogenolysis, a complex mixture being formed.

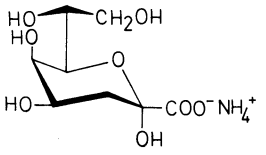
One method already described in the literature was used to give an ammonium salt of KDO from **6**[3]

Table 2. Conversion of **5a** into **6**



5a 6

Reagent	Temp	Time/h	Yield/%
KCN-MeOH	RT	5	7
K_2CO_3-MeOH	RT	5	0
MeONa-MeOH (2equiv, 0.03 M)	RT	5.5	28
MeONa-MeOH (0.2equiv, 0.003 M)	RT	5.5	0
MeONa-MeOH (10equiv, 0.15 M)	RT	5.5	0
MeONa-MeOH (2equiv, 0.03 M)	−20 °C	20	37
MeONa-MeOH (2equiv, 0.03 M)	−36 °C	40	26
MeONa-MeOH (0.2equiv, 0.03 M)	−20 °C	20	0
EtONa-EtOH (2equiv, 0.03 M)	−20 °C	20	45
PrONa-PrOH (2equiv, 0.03 M)	−20 °C	20	29



ammonium salt of KDO

Experimental

General Procedure. Melting points are uncorrected. NMR spectra were recorded in the solvents noted (Me_4Si, 0.00 ppm) with a JEOL FX 90A spectrometer. Column chromatography was performed by flash chromatography with silica gel. Specific rotations were measured with a JASCO DIP-360 polarimeter. IR spectra were recorded with a JASCO A-102 spectrometer.

2,3,4,5,6-Penta-*O*-acetyl-*aldehydo*-D-mannose. This compound was prepared as described by Miljikovic's method[12] from 2,3,4,5,6-penta-*O*-acetyl-*aldehydo*-D-mannose diethyl dithioacetal. The product was purified by column chromatography (ethyl acetate: hexane 1:5) as oil (it gradually crystallized). This compound could not be stored after purification, since it decomposed rapidly while in storage. ^1H NMR ($CDCl_3$) δ=2.00 (3H, s), 2.05 (3H, s), 2.10 (3H, s), 2.12 (3H, s), 2.17 (3H, s), 4.00—4.30 (2H, m), 4.90—5.25 (2H, m), 5.40—5.55 (2H, m), 9.42 (1H, d, J=1.0 Hz); IR (KBr) 1750, 1710 cm^{-1}.

Methyl α-(Dimethoxyphosphinyl)glycolate *t*-Butyldimethylsilyl Ether (1, R^1=*t*-butyldimethylsilyl). To a solution of methyl α-(dimethoxyphosphinyl)glycolate (0.198 g, 1.00 mmol) in DMF (1 ml) were added *t*-butyldimethylsilyl chloride (0.181 g, 1.20 mmol) and imidazole (0.082 g, 1.20 mmol) at room temperature. After stirring for 17 h at room temperature, the resulting mixture was extracted with diethyl ether; the product was purified by column chromatography (ethyl acetate: hexane 1:4) to give 0.255 g (82%) as oil; ^1H NMR ($CDCl_3$) δ=0.13 (6H, s), 0.93 (9H, s), 3.70 (3H, s), 3.77 (3H, s), 3.88 (3H, s), 4.58 (1H, d, J=18.0 Hz).

Methyl α-(Dimethoxyphosphinyl)glycolate Benzyl Ether (1, R^1=benzyl). To a solution of methyl α-(dimethoxyphosphinyl)glycolate (1.98 g, 10.0 mmol) in cyclohexane (60 ml) and dichloromethane (30 ml) were added benzyl trichloroacetimidate[13] (5.10 g, 20 mmol) and trifluoromethanesulfonic acid (0.44 ml, 5.0 mmol) at room temperature; the mixture was stirred for 23 h. The resulting mixture was extracted with dichloromethane and the product was purified by column chromatography (ethyl acetate:hexane 3:2) to give 2.73 g (95%) as oil; ^1H NMR ($CDCl_3$) δ=3.67 (3H, s), 3.73 (3H, s), 3.83 (3H, s), 4.37 (1H, d, J=18.4 Hz), 4.75 (1H, d, J=9.0 Hz), 4.83 (1H, d, J=9.0 Hz), 7.25 (5H, s).

Methyl 2-*O*-Acetyl-α-(dimethoxyphosphinyl)glycolate (1, R^1=acetyl). ^1H NMR ($CDCl_3$) δ=2.18 (3H, s), 3.73 (3H, s), 3.75 (3H, s), 3.92 (3H, s), 5.38 (1H, d, J=17.2 Hz).

Methyl 4,5,6,7,8-Penta-*O*-acetyl-2-*O*-(*t*-butyldimethylsilyl)-3-deoxy-D-*manno*-oct-2-enoate (5a). To a solution of methyl α-(dimethoxyphosphinyl)glycolate *t*-butyldimethylsilyl ether (0.225 g, 0.720 mmol) in THF (3 ml) was added 1 M lithium bis(trimethylsilyl)amide (1 M=1 mol dm^{-3}) in THF solution (0.660 ml) at −78 °C under an argon atmosphere. After 10 min, 2,3,4,5,6-penta-*O*-acetyl-*aldehydo*-D-mannose (0.234 g, 0.600 mmol) in THF (25 ml) solution was added dropwise over 20 min at −78 °C; the mixture was stirred for 2 h at −78 °C. The resulting mixture was extracted with diethyl ether and the products were separated by column chromatography (ethyl acetate:hexane 1:8). The faster moving compound was (*Z*)-**5a** (0.005 g, 1%) obtained as oil; ^1H NMR ($CDCl_3$) δ=0.20 (6H, s), 1.00 (9H, s), 1.99 (3H, s), 2.02 (3H, s), 2.04 (6H, s), 2.07 (3H, s), 3.72 (3H, s), 4.07—4.18 (1H, m), 5.00—5.22 (1H, m), 5.28—5.95 (5H, m); ^{13}C NMR ($CDCl_3$) δ=−4.2, 18.7, 20.4, 20.6, 20.7, 25.8, 52.3, 62.0, 65.5, 67.5, 68.1, 69.8, 114.1, 144.0, 169.4,

169.5, 169.8, 170.4. The slower moving compound was (*E*)-**5a** (0.231 g, 67%) obtained as colorless crystals; mp 56—59 °C. ^{1}H NMR ($CDCl_3$) δ=0.14 (6H, s), 0.92 (9H, s), 2.00 (3H, s), 2.04 (6H, s), 2.12 (3H, s), 2.16 (3H, s), 3.82 (3H, s), 4.06—4.21 (2H, m), 5.00—5.20 (1H, m), 5.28 (1H, d, J=9.8 Hz), 5.34 (1H, dd, J=2.1, 7.5 Hz), 5.50 (1H, dd, J=2.1, 9.0 Hz), 6.23 (1H, dd, J=7.5, 9.8 Hz); ^{13}C NMR ($CDCl_3$) δ=−5.0, 18.2, 20.6, 20.8, 20.9, 25.5, 52.0, 62.0, 67.7, 68.3, 69.9, 115.0, 145.6, 164.0, 169.4, 169.6, 169.7, 169.9, 170.5. IR (KBr) 1750, 1640, 1220 cm^{-1}; $[\alpha]_D^{28}$=31.2° (*c* 1.67, acetone); Found: C, 52.45, H, 7.11%. Calcd for $C_{25}H_{40}O_{13}Si$: C, 52.07, H. 6.99%. Other methyl 3-deoxy-D-*manno*-oct-2-enoate derivatives (**5**) were also obtained in a similar manner. ^{1}H NMR data of the main products are listed as follows.

Methyl 2,4,5,6,7,8-Hexa-*O*-acetyl-3-deoxy-D-*manno*-oct-2-enoate (5b); ^{1}H NMR ($CDCl_3$) δ=2.02 (12H, s), 2.08 (6H, s), 3.76 (3H, s), 3.73—4.18 (2H, m), 4.93—5.50 (1H, d, J=10.0 Hz), 6.22 (1H, dd, J=8.0, 10.0 Hz).

Methyl 4,5,6,7,8-Penta-*O*-acetyl-2-*O*-(2,2,2-trichloro-1,1-dimethylethoxycarbonyl)-3-deoxy-D-*manno*-oct-2-enoate (5c); ^{1}H NMR ($CDCl_3$) δ=2.02 (12H, s), 2.12 (3H, s), 3.82 (3H, s), 4.00—4.30 (2H, m), 5.00—5.60 (3H, m), 5.81 (1H, d, J=9.6 Hz), 6.23 (1H, dd, J=6.6, 9.6 Hz).

Methyl 4,5,6,7,8-Penta-*O*-benzyl-2-*O*-(*t*-butyldimethylsilyl)-3-deoxy-D-*manno*-oct-2-enoate (5d); ^{1}H NMR ($CDCl_3$) δ=0.13 (6H, s), 0.93 (9H, s), 3.58 (3H, s), 3.60—4.00 (5H, m), 4.20—4.70 (10H, m), 5.13 (1H, dd, J=2.4, 9.2 Hz), 5.58 (1H, d, J=9.2 Hz).

Methyl 4,5,6,7,8-Penta-*O*-benzyl-2-*O*-(2,2,2-trichloro-1,1-dimethylethoxycarbonyl)-3-deoxy-D-*manno*-oct-2-enoate (5e); ^{1}H NMR ($CDCl_3$) δ=3.66 (3H, s), 3.72—4.24 (5H, m), 4.40—4.82 (10H, m), 5.32 (1H, dd, J=5.0, 10.0 Hz), 6.15 (1H, d, J=10.0 Hz).

Methyl 4-*O*-Acetyl-2-*O*-(*t*-butyldimethylsilyl)-5,6:7,8-di-2-enoate (5f); ^{1}H NMR ($CDCl_3$) δ=3.68 (3H, s), 3.68—3.95 (5H, m), 4.25—4.78 (10H, m), 5.12 (1H, dd, J=2.5, 9.5 Hz), 5.32 (1H, d, J=9.5 Hz).

Methyl 4-*O*-acetyl-2-*O*-(*t*-butyldimethylsilyl)-5,6:7,8-di-*O*-isopropylidene-3-deoxy-D-*manno*-oct-2-enoate; ^{1}H NMR ($CDCl_3$) δ=0.17 (6H, s), 0.93 (9H, s), 1.35 (3H, s). 1.40 (9H, s), 2.03 (3H, s), 3.75 (3H, s), 3.75—4.33 (5H, m), 5.40 (1H, d, J=5.0 Hz), 6.22 (1H, dd, J=3.0, 5.0 Hz).

KDO 1,4-Lactone (6). To a solution of sodium ethoxide (0.106 g, 1.55 mmol) in ethanol (20 ml) was added **5a** (0.447 g, 0.775 mmol) in ethanol (32 ml) at −20 °C. This solution was stirred for 20 h at −20 °C. Then, Dowex 50 WX (H^+) was added at room temperature and the mixture stirred for 2.5 h. After filtration, the filtrate was evaporated under reduced pressure and ethanol–diethyl ether was added. Compound **6** was collected by filtration and dried to give 0.077 g (45%) of colorless crystals, mp 191—194 °C (lit,[3b] 192—194 °C). $[\alpha]_D^{28}$ +34.3° (*c* 1.4 water) (lit,[3b] $[\alpha]_D^{24}$ +31.8° (*c* 1.4, water).

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