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Note

Synthesis of 3-deoxy-6-thio-D-*manno*-2-octulosonic acid ("6-thio-KDO")*

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KDO is a constituent of the lipopolysaccharide of Gram-negative bacteria and links the polysaccharide and lipid A. The enzyme CMP-3-deoxyoctulosonate cytidyltransferase (CMP-KDO synthetase) is involved in the biosynthesis of the lipopolysaccharide and catalyses the formation of CMP-KDO from KDO and cytidine triphosphate. The activation of KDO before transfer to lipid A is believed to be the rate-limiting step in LPS biosynthesis[1].

In searching for antibacterial agents with specificity for Gram-negative bacteria, some substrate analogues of KDO as potential inhibitors of CMP-KDO synthetase have been synthesised[2,3] and we now report the synthesis of the title compound.

1,6-Anhydro-2,3-*O*-isopropylidene-β-D-talopyranose[4] (**1a**) was converted into the 4-triflate **1b** which, on treatment with sodium thiobenzoate in *N,N*-dimethylformamide, afforded the known[5] 4-thio compound **2** in high yield. Deprotection in two steps then gave 4-thio-D-mannose[5] (**5**), the 2,3:5,6-di-*O*-isopropylidene derivative of which was reduced to the alditol **8a**. *S*-*p*-Methoxybenzylation of **8a** yielded **8b**, which was oxidised to give the key intermediate 2,3:5,6-di-*O*-isopropylidene-4-*S*-(4-methoxybenzyl)-4-thio-*aldehydo*-D-mannose (**9**). Reaction of **9** with the sodium salt of dimethyl [methoxycarbonyl(trichloro-*tert*-butoxycarbonyloxy)methyl]phosphonate[6] yielded **10**. Treatment of **10** with zinc in acetic acid removed the trichloro-*tert*-butoxycarbonyl group to give **11** from which the *S*-protecting group was removed by oxidation with 2,3-dichloro-5,6-dicyanobenzoquinone to give **12**. Mild acid treatment of **12** then removed the isopropylidene groups to yield the ester **13**. Hydrolysis of **13** in 0.1M KOH and conversion of the product into the ammonium salt yielded the title compound **14**.

The 6-thio-KDO (ammonium salt) **14** did not inhibit CMP-KDO synthetase, possibly because only β-KDO is a substrate for the enzyme and **14** probably exists exclusively in its α form (equatorial carboxylic group). Only one epimer at C-2 was observed, but the configuration was not determined.

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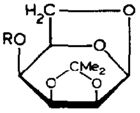
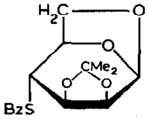
*Dedicated to Professor Hans Paulsen.



1a R = H

1b R = SO_2CF_3



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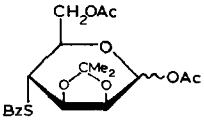

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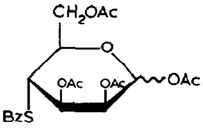

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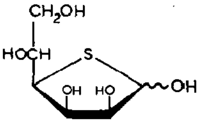

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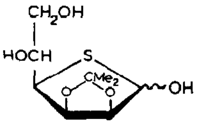

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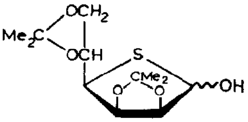

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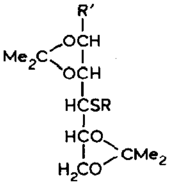
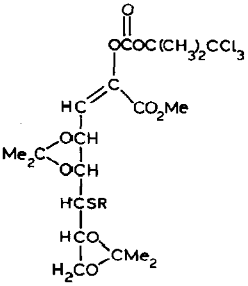


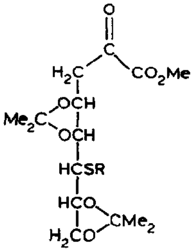
8a R = H, R′ = CH_2OH

8b R = 4-methoxybenzyl, R′ = CH_2OH

9 R = 4-methoxybenzyl, R′ = CHO

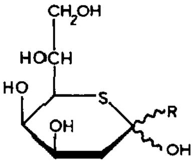


10 R = 4-methoxybenzyl



11 R = 4-methoxybenzyl

12 R = H (acyclic form depicted
cyclic form found)



13 R = CO_2Me

14 R = $CO_2^{-}\,{}^{+}NH_4$

EXPERIMENTAL

General methods. — These were the same as those described before[7]. Satisfactory elemental analyses for some of the syrupy compounds could not be obtained, but their homogeneity was established by chromatography and their structures by n.m.r. spectroscopy. ^{13}C-N.m.r. spectra (100 MHz) were obtained for solutions in $CDCl_3$ unless stated otherwise.

*1,6-Anhydro-2,3-*O*-isopropylidene-4-*O*-trifluoromethylsulfonyl-β-*D*-talopyranose* (**1b**). — To a stirred solution of trifluoromethanesulfonic anhydride (3.99 mL, 23.7 mmol) in dichloromethane (15 mL) at −15° were added dropwise a solution of pyridine (4.8 mL, 59.3 mmol) in dichloromethane (10 mL) followed by a solution of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-talopyranose[4] (**1a**; 4.0 g, 19.8 mmol) in dichloromethane (15 mL). The mixture was then stored for 30 min at −15°, when t.l.c. indicated that the reaction was complete. The mixture was diluted with dichloromethane, washed with aqueous 5% sodium hydrogencarbonate and water, dried, and concentrated. Column chromatography (silica gel; toluene–ethyl acetate, 4:1) of the residue gave **1b** (6.2 g, 94%), m.p. 79–81° (from dichloromethane–hexane), $[\alpha]_D^{22}$ −51° (*c* 1.3, chloroform). ^{13}C-N.m.r. data: δ 25.7 (CH_3), 25.9 (CH_3), 64.1, 71.0, 71.6, 76.7, 78.7 (C-4), 99.5 (C-1), 112.7 [$C(OMe)_2$].

Anal. Calc. for $C_{10}H_{13}F_3O_7S$: C, 35.9; H, 3.9; S, 9.6. Found: C, 35.1; H, 3.7; S, 9.7.

*1,6-Anhydro-2,3-*O*-isopropylidene-4-*S*-benzoyl-4-thio-β-*D*-mannopyranose* (**2**). — A mixture of **1b** (6.1 g, 18.35 mmol) and sodium thiobenzoate (7.38 g, 21.9 mmol) in *N,N*-dimethylformamide (30 mL) was heated for 1 h at 90°, then cooled to room temperature, and diluted with chloroform and water. The chloroform layer was washed with water, aqueous 5% sodium hydrogencarbonate, and water, dried, and concentrated. The crystalline residue was triturated with light petroleum, and filtered to yield **2** (5.3 g, 90%), m.p. 90–92°, $[\alpha]_D^{22}$ −62° (*c* 0.15, chloroform); lit.[5] m.p. 91–94°, $[\alpha]_D$ −61.9° (chloroform).

*1,6-Di-*O*-acetyl-4-*S*-benzoyl-2,3-*O*-isopropylidene-4-thio-*D*-mannopyranose* (**3**) *and 1,2,3,6-tetra-*O*-acetyl-4-*S*-benzoyl-4-thio-*D*-mannopyranose* (**4**). — A solution of **2** (5.3 g, 16.4 mmol) in a mixture (80 mL) of acetic anhydride, acetic acid, and sulfuric acid (70:30:1) prepared at 0° was stirred for 6 h at room temperature. Anhydrous sodium acetate (1.5 g) was added, the mixture was concentrated to dryness, and toluene was evaporated from the residue in order to remove traces of acetic acid and acetic anhydride. The residue was partitioned between chloroform and water, and the organic phase was washed with water, dried, and concentrated. Column chromatography (toluene–ethyl acetate, 4:1) of the oily residue gave **3** (60%) and **4** (29%). Compound **3** (4.2 g) had $[\alpha]_D^{22}$ +23° (*c* 2.6, chloroform). ^{13}C-N.m.r. data: δ 20.7 (2 OAc), 26.2 (CH_3), 27.8 (CH_3), 42.7 (C-4), 63.6, 73.7, 74.5, 91.0 (C-1), 110.2 [$C(OMe)_2$].

Anal. Calc. for $C_{20}H_{24}O_8S$: C, 56.6; H, 5.7; S, 7.6. Found: C, 57.1; H, 6.0; S, 6.5.

Compound **4** (2.2 g) had $[\alpha]_D^{22}$ +96° (*c* 2.4, chloroform); lit.[5] $[\alpha]_D$ +102° (chloroform).

*4-Thio-*D*-mannofuranose* (**5**) *and 2,3-O-isopropylidene-4-thio-*D*-mannofuranose* (**6**). — Methanolic 0.4M sodium methoxide (7 mL) was added at room temperature under nitrogen to a mixture of **3** (2.5 g, 5.89 mmol) and **4** (2.1 g, 4.48 mmol) in dry methanol (50 mL). The solution was stirred for 4–5 min, then neutralised with methanol-washed Dowex 50 (H^+) resin, filtered, and concentrated. Column chromatography (ethyl acetate–methanol, 8:2) of the residue gave **5** and **6**.

Compound **5** (0.64 g, 73% from **4**) had $[\alpha]_D^{22}$ +40° (*c* 0.15, water); lit.[5] $[\alpha]_D$ +41.8° (water). The 1,2,3,5,6-penta-acetate of **5**, prepared conventionally with acetic anhydride–pyridine followed by column chromatography (toluene–ethyl acetate 1:1), had $[\alpha]_D^{22}$ +144° (*c* 0.14 chloroform); lit.[5] $[\alpha]_D$ +148° (chloroform).

Compound **6** (1.05 g, 76% from **3**) had $[\alpha]_D^{22}$ +46° (*c* 0.4, methanol). ^{13}C-N.m.r. data: δ 24.8 (CH_3), 26.0 (CH_3), 52.6 (C-4), 70.7, 82.6, 85.4, 89.3, 111.2 [$C(OMe)_2$].

*2,3:5,6-Di-O-isopropylidene-4-thio-*D*-mannofuranose* (**7**). — A catalytic amount of toluene-*p*-sulfonic acid and 2,2-dimethoxypropane (0.75 mL, 6.2 mmol) were added at room temperature to a solution of **5** (0.64 g, 3.1 mmol) and **6** (1 g, 4.23 mmol) in dry acetone (20 mL). The mixture was stirred for 1 h, triethylamine (5 mL) was then added, and the solution was concentrated. The residue was partitioned between chloroform and water, and the organic layer was washed with saturated aqueous sodium chloride and water, dried, and concentrated. Column chromatography (toluene–ethyl acetate, 1:1) of the residue and crystallisation of the product from toluene gave **7** (1.6 g, 80%), m.p. 121–123°, $[\alpha]_D^{22}$ +56° (*c* 1, chloroform). ^{13}C-N.m.r. data: δ 24.6, 25.4, 25.8, 26.9 (4 CH_3), 54.7 (C-4), 67.9 (C-6), 73.9, 82.3, 85.2, 89.3, 109.2, 110.1 [2$C(OMe)_2$].

Anal. Calc. for $C_{12}H_{20}O_5S$: C. 52.2; H, 7.3; S, 11.6. Found: C, 52.3; H, 7.4; S, 11.3.

*2,3:5,6-Di-O-isopropylidene-4-thio-*D*-mannitol* (**8a**). — Sodium borohydride (0.86 g, 22.8 mmol) was added at room temperature to a solution of **7** (2.1 g, 7.6 mmol) in aqueous 80% ethanol (140 mL). After 3 h, the same amount of sodium borohydride was added. This procedure was repeated several times until t.l.c. indicated that the reaction was complete. Aqueous acetic acid was then added dropwise until all gas evolution ceased, the residue was partitioned between ether and water, and the ether phase was washed with water and concentrated. Column chromatography (toluene–ethyl acetate, 2:1) of the residue gave **8a** (1.6 g, 75%), $[\alpha]_D^{22}$ −18° (*c* 0.8, water). ^{13}C-N.m.r. data (100 MHz, $CDCl_3$): δ 25.1, 25.6, 26.8 (4 CH_3), 42.6 (C-4), 61.3 (C-1), 68.7 (C-6), 75.7, 78.2, 78.5, 108.4, 110.2 [2$C(OMe)_2$].

Anal. Calc. for $C_{12}H_{22}O_5S$: C, 51.8; H, 8.0; S, 11.5. Found: C, 51.9; H, 8.1; S, 11.3.

*2,3:5,6-Di-O-isopropylidene-4-S-(4-methoxybenzyl)-4-thio-*D*-mannitol* (**8b**). — A solution of **8a** (660 mg, 2.38 mmol) in *N,N*-dimethylformamide (20 mL) was stirred with sodium hydride (4.83 mmol, 55% suspension in oil) at −10° under nitrogen. After 5 min, *p*-methoxybenzyl chloride (0.32 mL, 2.38 mmol) was added and the mixture was stirred for 15 min whilst the temperature rose to room temperature. Methanol was added dropwise to the solution until all gas evolution ceased. The mixture was diluted with dichloromethane, washed with water, saturated aqueous sodium hydrogencarbonate, and water, dried, and concentrated. Column chromatography (toluene–ethyl acetate, 3:1) of the residue gave **8b** (800 mg, 84%), $[\alpha]_D^{22}$ +68° (*c* 0.9, chloroform). ^{13}C-N.m.r. data: δ 25.5, 26.3, 27.8 (4 CH_3), 36.2 (CH_2), 45.1 (C-4), 55.3 (OCH_3), 61.5 (C-1), 69.3, 77.1, 78.4, 79.8.

Anal. Calc. for $C_{20}H_{30}O_6S$: C, 60.3; H, 7.6; S, 8.1. Found: C, 60.3; H, 7.6; S, 8.5.

*2,3:5,6-Di-O-isopropylidene-4-S-(4-methoxybenzyl)-4-thio-*aldehydo-D-*mannose* (**9**). — A solution of **8b** (0.8 g, 2.0 mmol) in anhydrous methyl sulfoxide and acetic anhydride (5:1, 12 mL) was stored for 16 h at room temperature and then lyophilised. Column chromatography (toluene–ethyl acetate, 2:1) of the residue gave **9** (0.68 g, 86%), $[\alpha]_D^{22}$ −35° (*c* 0.5, chloroform). ^{13}C-N.m.r. data: δ 24.4, 25.9, 26.8 (4 CH_3), 38.5 (CH_2), 49.2 (C-4), 68.0 (C-6), 77.8, 80.7, 82.1, 109.4, 110.7 [2 $C(OMe)_2$].

Anal. Calc. for $C_{20}H_{28}O_6S$: C, 60.6; H, 7.1; S, 8.1. Found: C, 59.9; H, 7.1; S, 8.8.

*Methyl 3-deoxy-4,5:7,8-di-O-isopropylidene-6-S-(4-methoxybenzyl)-6-thio-*D-manno-*octulosonate* (**11**). — A solution of dimethyl [methoxycarbonyl(trichloro-*tert*-butoxycarbonyloxy)methyl]phosphonate[6] (2.53 g, 6.56 mmol) in tetrahydrofuran (4 mL) was added *dropwise* to a stirred solution of lithium hexamethyldisilylamide (8.2 mmol) in dry, freshly distilled tetrahydrofuran (4 mL) containing 4Å molecular sieve, at −78° under nitrogen. The mixture was stirred for 10 min and a solution of **9** (0.65 g, 1.64 mmol) in tetrahydrofuran (8 mL) was then added dropwise. After 10 min, the mixture was allowed to warm slowly to 0°, then stirred for 20 min, diluted with ether (50 mL), washed with aqueous 5% hydrochloric acid, water, saturated aqueous sodium hydrogencarbonate, and water, dried, and concentrated. Column chromatography (toluene–ethyl acetate, 4:1) of the residue afforded **10** (0.84 g, 76%), $[\alpha]_D^{22}$ −89° (*c* 0.2, chloroform). ^{13}C-N.m.r. data: δ 20.9, 21.0 [2 $C(\mathit{C}H_3)_2$], 24.1, 27.4, 25.9, 26.7 (4 CH_3), 37.5 (CH_2), 48.9 [$O\mathit{C}(CH_3)_2$], 50.5 (C-4), 52.5 ($\mathit{C}H_3OCO$), 55.2 ($\mathit{C}H_3OC_6H_4$), 67.8 (C-6), 74.3, 78.8, 80.3, 91.2 (CCl_3), 109.1, 109.3 [2$C(OMe)_2$].

A solution of **10** (0.83 g, 1.24 mmol) in aqueous 3% glacial acetic acid in ether (20 mL) was stirred with zinc dust (1.62 g, 24.7 mmol) for 1 h at room temperature then filtered, washed with saturated aqueous sodium hydrogencarbonate and water, dried, and concentrated. Column chromatography (toluene–ethyl acetate, 4:1) of the residue yielded syrupy **11** (0.32 g, 55%), $[\alpha]_D^{22}$ +96° (*c* 0.15, chloroform). ^{13}C-N.m.r. data: δ 25.4, 26.3, 27.4 (4 CH_3), 36.0 (CH_2), 40.9 (C-3), 45.5 (C-6), 52.9 ($\mathit{C}H_3OCO$), 55.2 ($\mathit{C}H_3OC_6H_4$), 69.1, 74.2, 77.2, 80.0, 108.1, 109.8 [2$C(OMe)_2$].

Anal. Calc. for $C_{23}H_{32}O_8S$: C, 59.0; H, 6.9; S, 6.8. Found: C, 58.9; H, 6.8; S, 6.6.

*Methyl 3-deoxy-4,5:7,8-di-O-isopropylidene-6-thio-*D-manno-*2-octulopyrano-*

sonate (**12**). — 2,3-Dichloro-5,6-dicyanobenzoquinone (0.16 g, 0.71 mmol) was stirred with a solution of **11** (0.3 g, 0.64 mmol) in dichloromethane and water (18:1, 10 mL) for 2 h at room temperature, then filtered, and concentrated. Column chromatography (toluene–ethyl acetate, 3:1) of the residue gave **12** (0.16 g, 72%), $[\alpha]_D^{22}$ +47° (*c* 0.3, methanol). ^{13}C-N.m.r. data (CD_3OD): δ 25.7, 26.4, 26.9, 27.6 (4 CH_3), 38.0 (OCH_2), 45.1 (C-3), 53.5, 68.2, 71.9, 73.7, 76.2, 82.5 (C-2), 109.9, 110.3 [2C(OMe)$_2$], 172.8 (C-1).

Anal. Calc. for $C_{15}H_{24}O_7S$: C, 51.7; H, 6.9; S, 9.2. Found: C, 51.6; H, 6.7; S, 8.2.

*Methyl 3-deoxy-6-thio-*D-manno-*2-octulopyranosonate* (**13**). — A solution of **12** (0.15 g, 0.43 mmol) in methanol (5 mL) was stirred with toluene-*p*-sulfonic acid (0.074 g, 0.43 mmol) for 30 min at room temperature when t.l.c. indicated that the reaction was complete. Column chromatography (ethyl acetate–methanol, 4:1) of the mixture gave **13** (0.055 g, 46%), $[\alpha]_D^{22}$ +27° (*c* 0.3, methanol). ^{13}C-N.m.r. data (D_2O): δ 38.0 (OCH_3), 47.1 (C-3), 55.2, 65.1, 67.4, 69.1, 71.4, 83.1 (C-2), 174.1 (C-1).

*Ammonium 3-deoxy-6-thio-*D-manno-*2-octulopyranosonate (6-thio-KDO)* (**14**). — A solution of **13** (40 mg, 0.149 mmol) in 0.1M KOH (2.0 mL) was kept for 4 h at room temperature. T.l.c. (ethyl acetate–methanol–acetic acid–water, 3:2:1:1) then indicated that hydrolysis was complete. The solution was passed through a column of Dowex 50 ($^+NH_4$) resin, by elution with water (5 mL), and lyophilised to yield **14** (40 mg, 99%), $[\alpha]_D^{22}$ +101.2° (*c* 0.5, water). N.m.r. data (D_2O): ^{13}C, δ 38.5 (C-3), 46.6 (C-6), 64.6 (C-8), 67.3, 69.3, 70.9, 85.0 (C-2), 177.9 (C-1); ^{1}H (400 MHz), δ 2.01 (dd, $J_{3e,3a}$ 13.2, $J_{3e,4}$ 4.4 Hz, H-3*e*), 2.31 (dd, $J_{3a,4}$ 12.4 Hz, H-3*a*), 3.37 (dd, $J_{6,7}$ 9.28 Hz, H-6), 3.57 (dd, $J_{8,8'}$ 12.2 Hz, H-8), 3.77 (dd, H-8), 3.82 (ddd, $J_{7,8}$ 2.93, $J_{7,8}$ 6.10 Hz, H-7), 3.93 (ddd, $J_{4,5}$ 2.69 Hz, H-4), 4.27 (dd, $J_{5,6}$ 1.47 Hz, H-5).

Anal. Calc. for $C_8H_{17}NO_7S \cdot H_2O$: C, 33.2; H, 6.6; N, 4.8; S, 11.1. Found: C, 33.8; H, 6.6; N, 4.6; S, 11.3.

ACKNOWLEDGMENTS

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