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Note

6-*O*-Glycosylation of a lipid A-subunit analog (GLA-27) with methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosyl bromide)onate (a KDO derivative)

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3-Deoxy-D-*manno*-2-octulosonic acid (KDO) is a prominent constituent of the cell-surface macromolecules of Gram-negative bacteria[1], and recently has also been found[2] as a component of the cell walls of higher plants. In the bacterial lipopolysaccharides (LPS), KDO occurs as an α-D-ketosidic component linked at O-6′ of the D-glucosamine-disaccharide backbone of[3–5] lipid A, but its distinct biological roles in LPS are still obscure.

In the course of a synthetic approach[6–10] designed to clarify the relationship of the molecular structure and the biological activity of bacterial lipid A, and to obtain new sources of nontoxic biological-response modifiers (BRM), we have found[11] that a 4-*O*-phosphono-D-glucosamine derivative named[6,7] GLA-27 distinctly shows some of the biological activities expressed by lipid A. In addition, it was also suggested[12] that the activities could be selectively expressed by modifying the lipophilic structure of GLA-27.

We now describe the 6-*O*-glycosylation of a diastereoisomeric pair, namely, 2-deoxy-4-*O*-phosphono-3-*O*-tetradecanoyl-2-[(3*R*)- and (3*S*)-3-tetradecanoyloxy tetradecanamido]-D-glucose (GLA-27-*R* and GLA-27-*S*)[7], with methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α-D-*manno*-2-octulopyranosyl bromide)onate[13] (**2**).

Condensation of **1***R* or **1***S*, which is a synthetic intermediate[7] of GLA-27, with the bromide **2** was accomplished by use of $Hg(CN)_2$ and $HgBr_2$ as the catalysts. The resulting **3***R* or **3***S* was a mixture of the α- (70–80%) and β- (20–30%) glycosides, which were separated by chromatography after hydrogenolytic removal of the benzyl group at O-1. The anomeric configurations of **4***R*(α) and **4***R*(β) thus obtained were initially assigned on the basis of the glycosylation yields and the optical rotations. In their ^{1}H-n.m.r. spectra, the C-8 methylene protons of the KDO residue appeared at δ 4.00 and 4.59 for the major product, and at 4.24 and 4.36 for

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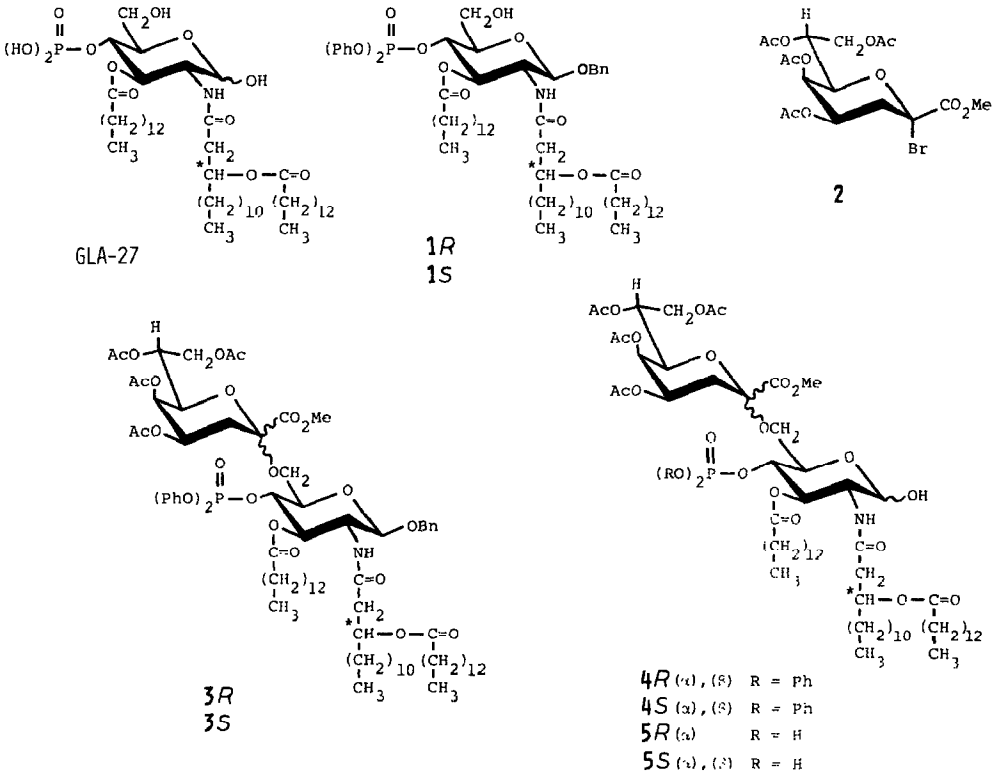

the minor product, respectively, showing the characteristic separation patterns in the chemical shifts for the α-and β-ketoside derivatives[14] of KDO. Similarly, their isomers **4***S*(α) and **4***S*(β) were also assigned to the corresponding ketosides. Finally, the phenyl groups of **4***R*(α), **4***S*(α), or **4***S*(β) were removed by hydrogenolysis, to give the desired product **5***R*(α), **5***S*(α), or **5***S*(β) respectively, in nearly quantitative yield.

EXPERIMENTAL

General methods. — See ref. 7.

*Benzyl 2-deoxy-4-*O*-(diphenylphosphono)-6-*O*-[methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-*α*,*β*-*D*-manno-2-octulopyranosyl)onate]-3-*O*-tetradecanoyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-*β*-*D*-glucopyranoside* (**3R**). — To a solution of **1***R* (0.21 g) in dry dichloromethane (4.5 mL) were added $Hg(CN)_2$ (78 mg), $HgBr_2$ (30 mg), and molecular sieves 4 A (0.28 g); the mixture was stirred for 3 h, and then the bromide **2** (0.13 g), dissolved in a small amount of dichloromethane, was added. After completion of the reaction (t.l.c., 5:2 chloroform–ether or 1:1 ethyl acetate–hexane), the suspension was filtered through Celite, and the solid washed with dichloromethane. The filtrate and washings were combined, washed with

aqueous potassium iodide, dried, and evaporated. The residue was chromatographed on a column of silica gel (Wakogel C-300) with 2:1 hexane–ethyl acetate, to give a syrup of **3***R* (0.238 g; 84%); the ^{1}H-n.m.r. spectrum showed that ~80% of **3***R* was the α-disaccharide; $[\alpha]_D$ +4.6° (*c* 0.9, chloroform); ν_{max}^{film} 3320 (NH), 1750 (ester), 1660, 1540 (amide), 960 (P-O-Ph), and 780–690 cm^{-1} (Ph); ^{1}H-n.m.r. data for the α-disaccharide (270 MHz, $CDCl_3$): δ 0.88 (~t, 9 H, Me), 1.0–1.45, 1.5–1.7 (m, 64 H, –C*H*$_2$–), 1.86, 1.97, 2.07, 2.08 (4 s, 12 H, MeCO), 1.8–2.5 (m, 8 H, –COC*H*$_2$– and H-3′), 3.63 (s, 3 H, CO_2Me), 4.25 (dd, 1 H, $J_{6',7'}$ ~10, $J_{5',6'}$ 1.5 Hz, H-6′), 5.00 (m, 1 H, H-3 of the 3-hydroxytetradecanoyl group), 5.18 (m, 1 H, $J_{7',8'}$ 4.4, 2.6 Hz, H-7′), 5.9 (d, 1 H, N*H*), and 7.05–7.45 (m, 15 H, Ph).

Anal. Calc. for $C_{84}H_{128}NO_{23}P$ (1550.85): C, 65.05; H, 8.32; N, 0.90. Found: C, 65.31; H, 8.24; N, 0.87.

*Benzyl 2-deoxy-4-*O*-(diphenylphosphono)-6-*O*-[methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α,β-*D*-manno-2-octulopyranosyl)onate]-3-*O*-tetradecanoyl-2-[(3S)-3-tetradecanoyloxytetradecanamido]-β-*D*-glucopyranoside* (**3S**). — Compound **1***S* (0.2 g) was treated with **2** (0.13 g) as just described, to afford **3***S* (0.261 g; 92%); the ^{1}H-n.m.r. spectrum showed that 70–80% of **3***S* was the α-disaccharide; ν_{max}^{film} 3320 (NH), 1750 (ester), 1660, 1550 (amide), 960 (P–O–Ph), and 780–690 cm^{-1} (Ph). The pure α-disaccharide was obtained by rechromatography; $[\alpha]_D$ +9.2° (*c* 0.6, chloroform), and the ^{1}H-n.m.r. data were similar to those assigned for the α-disaccharide **3***R*.

Anal. Found: C, 65.28; H, 8.16; N, 0.93.

*2-Deoxy-4-*O*-(diphenylphosphono)-6-*O*-[methyl (4,5,7,8-tetra-*O*-acetyl-3-deoxy-α- and -β-*D*-manno-2-octulopyranosyl)onate]-3-*O*-tetradecanoyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-*D*-glucose* [**4R**(α) *and* **4R**(β)]. — To a solution of **3***R* (0.2 g) in ethanol was added palladium-black catalyst prepared from palladium chloride (95 mg), and the mixture was stirred for 2 h under hydrogen. After completion of the reaction (t.l.c., 1:1 hexane–ethyl acetate), the catalyst was filtered off, and washed with a mixture of methanol, ethanol, and chloroform. The filtrate and washings were combined, and evaporated to a syrup that was chromatographed on a column of silica gel with 2:1 hexane–ethyl acetate, to give **4***R*(α) (0.131 g; 70%) and **4***R*(β) (17.5 mg; 9.3%), respectively, as syrups.

Compound **4***R*(α) had $[\alpha]_D$ +38° (*c* 1, chloroform); ν_{max}^{film} 3450 (OH), 3390 (NH), 1750 (ester), 1660, 1540 (amide), 960 (P–O–Ph), and 780–690 cm^{-1} (Ph); ^{1}H-n.m.r. data (270 MHz, $CDCl_3$): δ 1.96, 1.97, 2.06, 2.07 (4 s, 12 H, MeCO), 3.65 (s, 3 H, CO_2Me), 3.68, 3.92 (2 dd, 2 H, J_{gem} 11–12 Hz, H-6), 4.00 (dd, 1 H, J_{gem} 12, $J_{7',8'}$ 5 Hz, H-8a′), 4.18 (~d, 1 H, $J_{6',7'}$ 9–10 Hz, H-6′), 4.29 (m, 1 H, H-5), 4.58 (~q, 1 H, $J_{3,4} = J_{4,5} = J_{4,P}$ = 9–10 Hz, H-4), 4.59 (dd, 1 H, $J_{7',8'}$ 3 Hz, H-8b′), 5.05–5.2 (m, 2 H, H-3 of the 3-hydroxytetradecanoyl group and H-7′), 5.23 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 5.29 (m, 1 H, H-5′), 5.37 (m, $J_{3',4'}$ ~12 and 5, $J_{4',5'}$ 3 Hz, H-4′), 5.45 (dd, 1 H, $J_{2,3}$ 10.6, $J_{3,4}$ 9.2 Hz, H-3), 6.15 (d, 1 H, N*H*), and 7.05–7.4 (m, 10 H, Ph).

Anal. Calc. for $C_{77}H_{122}NO_{23}P$ (1460.74): C, 63.31; H, 8.42; N, 0.96. Found: C, 63.49; H, 8.25; N, 1.00.

Compound **4***R*(β) had $[\alpha]_D$ +26° (*c* 0.3, chloroform); ν_{max}^{film} 3400 (OH), 3350 (NH), 1750 (ester), 1660, 1540 (amide), 960 (P–O–Ph), and 780–690 cm^{-1} (Ph); ^{1}H-n.m.r. data: δ 1.980, 1.983, 2.04, 2.10 (4 s, 12 H, MeCO), 3.68 (s, 3 H, CO_2Me), 3.67 (dd, 1 H, H-6a), 4.03–4.1 (m, 2 H, H-6b,6'), 4.1–4.3 (m, 2 H, H-2,5), 4.24 (dd, 1 H, J_{gem} 12, $J_{7',8'}$ 4.4 Hz, H-8a'), 4.36 (dd, 1 H, $J_{7',8'}$ 2 Hz, H-8b'), 4.65 (~q, 1 H, H-4), 4.88 (m, 1 H, H-4'), 5.05–5.2 (m, 2 H, H-3 of the 3-hydroxytetradecanoyl group and H-7'), 5.46 (dd, 1 H, H-3), 6.02 (d, 1 H, N*H*), and 7.05–7.4 (m, 10 H, Ph).

Anal. Found: C, 63.27; H, 8.40; N, 0.98.

*2-Deoxy-4-O-(diphenylphosphono)-6-O-[methyl (4,5,7,8-tetra-O-acetyl-3-deoxy-α- and -β-*D*-manno-2-octulopyranosyl)onate]-3-O-tetradecanoyl-2-[(3S)-3-tetradecanoyloxytetradecanamido]-*D*-glucose* [**4**S(α) *and* **4**S(β)]. — Hydrogenolytic removal of the benzyl group of **3***S* (0.295 g) as just described for **3***R* afforded **4***S*(α) (0.167 g; 60%) and **4***S*(β) (58 mg; 21%), respectively, as syrups.

Compound **4***S*(α) had $[\alpha]_D$ +39° (*c* 0.6, chloroform); ν_{max}^{film} 3400 (OH), 3360 (NH), 1740 (ester), 1660, 1550 (amide), 950 (P–O–Ph), and 780–680 cm^{-1} (Ph); ^{1}H-n.m.r. data (270 MHz, $CDCl_3$): δ 1.95, 1.96, 2.04, 2.06 (4 s, 12 H, MeCO), 3.64 (s, 3 H, CO_2Me), 3.66, 3.91 (2 dd, 2 H, H-6), 4.02 (dd, 1 H, H-8a'), 4.16 (m, 1 H, H-2), 4.21 (dd, 1 H, H-6'), 4.30 (m, 1 H, H-5), 4.57 (~q, 1 H, H-4), 4.57 (dd, 1 H, H-8b'), 5.05–5.2 (m, 2 H, H-3 of the 3-hydroxytetradecanoyl group and H-7'), 5.21 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 5.28 (m, 1 H, H-5'), 5.34 (m, 1 H, H-4'), 5.45 (dd, 1 H, H-3), 6.16 (d, 1 H, N*H*), and 7.05–7.4 (m, 10 H, Ph); the coupling constants were similar to those of **4***R*(α).

Anal. Found: C, 63.20; H, 8.38; N, 0.96.

Compound **4***S*(β) had $[\alpha]_D$ +30° (*c* 1, chloroform); ν_{max}^{film} 3400 (OH), 3350 (NH), 1740 (ester), 1660, 1530 (amide), 950 (P–O–Ph), and 770–680 cm^{-1} (Ph); ^{1}H-n.m.r. data (270 MHz, $CDCl_3$): δ 4.25 (dd, 1 H, J_{gem} 12, $J_{7',8'}$ 4–5 Hz, H-8a'), and 4.36 (dd, 1 H, $J_{7',8'}$ 2.2 Hz, H-8b').

Anal. Found: C, 63.52; H, 8.27; N, 0.88.

*2-Deoxy-6-O-[methyl (4,5,7,8-tetra-O-acetyl-3-deoxy-α-*D*-manno-2-octulopyranosyl)onate]-4-O-phosphono-3-O-tetradecanoyl-2-[(3R)-3-tetradecanoyloxytetradecanamido]-*D*-glucose* [**5**R(α)]. — A solution of **4***R*(α) (91 mg) in ethanol was stirred with prereduced, Adams' platinum catalyst in a hydrogen atmosphere. After completion of the reaction (t.l.c., 4:1 chloroform–methanol), the catalyst was filtered off, and washed well with methanol–ethanol; the filtrate and washings were combined, and evaporated. The product was purified by chromatography on a short column of silica gel (Wakogel C-200), to give **5***R*(α) in nearly quantitative yield. Compound **5***R*(α) gave a positive test with a spray reagent[18] specific for the phosphono group; $[\alpha]_D$ +32° (*c* 0.3, chloroform); ν_{max}^{film} 3450 (OH), 3360 (NH), 1750 (ester), 1660, 1550 (amide), and complete loss of the peaks at 960 (P–O–Ph) and 800–650 cm^{-1} (Ph); ^{1}H-n.m.r. data (270 MHz, $CDCl_3$): δ 0.88 (~t, 9 H, Me), 1.0–

1.4, 1.4–1.7 (m, 64 H, $-CH_2-$), 1.99, 2.01, 2.08, 2.09 (4 s, 12 H, MeCO), 1.8–2.5 (m, 8 H, H-3′ and $-COCH_2-$), 3.81 (s, 3 H, CO_2Me), 4.60 (dd, 1 H, H-8′), 5.18 (d, 1 H, *J* 3.3 Hz, H-1), 6.28 (d, 1 H, *J* 8.8 Hz, N*H*), and complete loss of the phenyl protons; other protons appeared between δ 3.55–5.45.

Anal. Calc. for $C_{65}H_{114}NO_{23}P$ (1308.55): C, 59.66; H, 8.78; N, 1.07. Found: C, 59.42; H, 8.86; N, 1.13.

2-Deoxy-6-O-[methyl (4,5,7,8-tetra-O-acetyl-3-deoxy-α-D-manno-2-octulopyranosyl)onate]-4-O-phosphono-3-O-tetradecanoyl-2-[(3S)-3-tetradecanoyloxytetradecanamido]-D-glucose [5S(α)]. — Hydrogenolysis of the phenyl groups of **4***S*(α) (0.134 g) was performed as described for the preparation of **5***R*(α), to afford **5***S*(α) (0.12 g) as a syrup, $[\alpha]_D$ +45° (*c* 0.8, chloroform); ν_{max}^{KBr} 3450 (OH), 3360 (NH), 1750 (ester), 1660, 1550 (amide), and complete loss of the peaks at 960 (P–O–Ph) and 800–650 cm^{-1} (Ph); ^{1}H-n.m.r. data were similar to those of **5***R*(α).

Anal. Found: C, 59.48; H, 8.62; N, 1.04.

2-Deoxy-6-O-[methyl (4,5,7,8-tetra-O-acetyl-3-deoxy-β-D-manno-2-octulopyranosyl)onate]-4-O-phosphono-3-O-tetradecanoyl-2-[(3S)-3-tetradecanoyloxytetradecanamido]-D-glucose [5S(β)]. — Compound **5***S*(β) (42 mg) was obtained from **4***S*(β) (50 mg) as a syrup, $[\alpha]_D$ +35° (*c* 0.6, chloroform); ν_{max}^{film} 3400 (OH), 3370 (NH), 1750 (ester), 1660, 1550 (amide), and complete loss of bands at 960 (P–O–Ph) and 800–650 cm^{-1} (Ph); ^{1}H-n.m.r. data (270 MHz, $CDCl_3$): δ 0.88 (~t, 9 H, Me), 1.0–1.4, 1.4–1.7 (m, 64 H, $-CH_2-$), 1.99, 2.02, 2.11, 2.17 (4 s, 12 H, MeCO), 2.0–2.5 (m, 8 H, H-3′ and $-COCH_2-$), 3.83 (s, 3 H, CO_2Me), 4.51 (dd, 1 H, J_{gem} 12.8, $J_{7',8'}$ 4 Hz, H-8′), 4.87 (m, 1 H, H-4′), 5.40 (~t, 1 H, H-3), 6.35 (d, 1 H, *J* 8.8 Hz, N*H*), and complete loss of the phenyl protons.

Anal. Found: C, 59.84; H, 8.75; N, 1.10.

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