SYNTHESIS OF THE SEQUENCE OF HEPT-(α1→5)-KDO-(α2→6)-D-GLUCOSAMINE-4-PHOSPHATE OF LIPOPOLYSACCHARIDE[1)]

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We have synthesized Hept-(α1→5)-KDO-(α2→6)-D-glucosamine-4-phosphate (1), which is located in the inner core and lipid A regions of lipopolysaccharide. Compound 1 was mitogenic.

KEYWORDS glucosamine-4-phosphate; KDO; heptose; lipid A; LPS; mitogenicity

Lipopolysaccharide (LPS) isolated from the cell walls of various Gram-negative bacteria, is composed of four structural regions: O-polysaccharide, outer core, inner core, and a lipophilic portion of LPS, lipid A.[2)] The inner core region of LPS consists of 3-deoxy-D-manno-2-octulosonic acid (KDO) and L-glycero-D-manno-heptose. Lipid A consists as a central backbone of a 1,6-linked β-D-glucosamine disaccharide substituted by phosphate groups and by ester- and amide-bound fatty acids.[3)] The KDO region of LPS is bound to lipid A through (α2→6) linkage, and the heptose region is bound to KDO through (α1→5) linkage, as shown in chart 1. The KDO as a ketosidic component in LPS plays a biologically important role in being mitogenic and in amplifying the antitumor activity of lipid A.[4)] The heptose region in the inner-core oligosaccharide seems to be an inducer in immunological reactions,[5)] although its biological roles in LPS are not entirely clear.[6)]

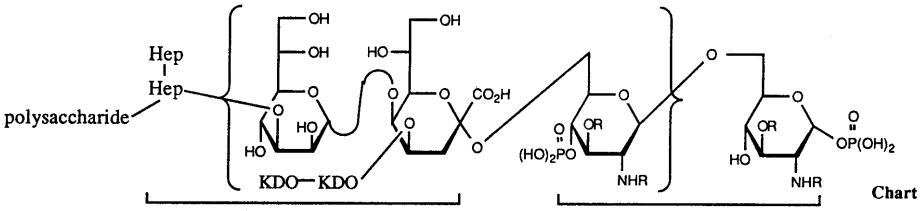
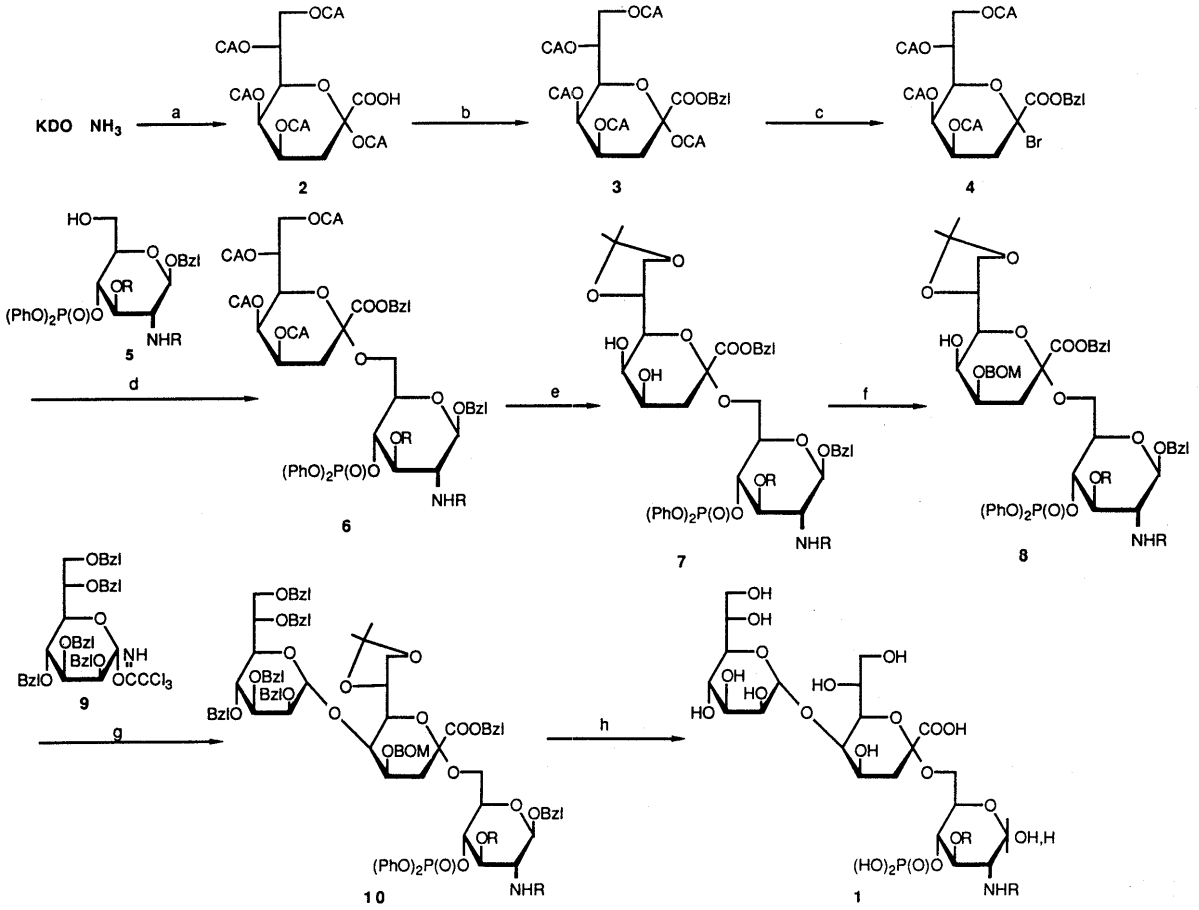


Chart . 1

In the course of investigations[7)] on the relationship between the molecular structure and the biological activity of the nonreducing-sugar subunit analogs of lipid A, we demonstrated that some α(2→6)-linked KDO-acylated 4-O-monophosphorylglucosamine derivatives have mitogenic activity comparable to that of lipid A.[7c,d,e)] Also, it is strongly suggested that the heptosyl-KDO linkage is necessary for the expression of the common LPS specifity.[5b)] Here we wish to report the synthesis of Hept-(α1→5)-KDO-(α2→6)-D-glucosamine-4-phosphate (1), in order to examine the biological significance of KDO and heptose residues in the inner-core region of bacterial lipopolysaccharide.

To synthesize KDO-(α2→6)-GlcN derivative (8), the hydroxyl groups of KDO·NH_3 were treated with chloroacetic anhydride, pyridine, and 4-dimethylaminopyridine in CH_3CN to give the per-O-chloroacetylated compound (2) [39%, mp 121-124℃, $[\alpha]_D^{24}$ +50.2° (c=0.99, $CHCl_3$)]. The carboxyl group of 2 was esterified with phenyldiazomethane to give the fully protected compound (3) [53%, syrup, $[\alpha]_D^{21}$ +51.5° (c=0.99, $CHCl_3$)]. Treatment of 3 with titanium tetrabromide in CH_2Cl_2-AcOEt(10:1) gave the 2-bromo compound (4) in nearly quantitative yield. The unstable bromide (4) was used for the subsequent glycosylation without further purification. Glycosylation of the glycosyl acceptor (5), prepared from benzyl 2-amino-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranoside in 5 steps,[7d,f)] with the glycosyl donor 4 in the presence of $Hg(CN)_2$, $HgBr_2$ and Molecular Sieves 4A in CH_2Cl_2 at room temperature for 48 h gave the desired α-linked disaccharide containing KDO (6) [40%, syrup, $[\alpha]_D^{20}$ +5.1° (c=0.99, $CHCl_3$)]. The α-D-anomeric configuration of the KDO residue was ascertained by the chemical shift value (δ 5.44) of the signal attributable to H-4, which is indicative of the α-D-anomeric configuration of per-O-chloroacetylated KDO derivatives.[8)] Selective removal of the

chloroacetyl groups of 6 was carried out with hydrazinedithiocarbonate[9] in 2,6-lutidine-AcOH (3:1) at 0° for 2 h and then treated with 2-methoxypropene[10] in the presence of a catalytic amount of p-toluenesulfonic acid in DMF to give the isopropylidene compound (7) [52%, syrup, $[\alpha]_D^{21}$ +5.5° (c=1.00)]. An equatorially oriented hydroxyl group of the C-4 of the KDO moiety of 7 was regioselectively protected with benzyloxymethyl chloride and pyridine in CH_2Cl_2 to give the benzyloxymethyl compound (8) [84%, syrup, $[\alpha]_D^{20}$ +6.1° (c=1.40, $CHCl_3$)]. Coupling of 8 and the suitably blocked L-glycero-D-manno-heptose derivative (9)[11] was carried out in the presence of a catalytic amount of p-toluenesulfonic acid to give the α-linked trisaccharide (10) as a single anomer [55%, $[\alpha]_D^{20}$ +7.7° (c=1.66, $CHCl_3$), ^{1}H-NMR:δ 5.26 (1H, br s, H-1 of heptose moiety), and ^{13}C-NMR:δ 100.5 (C-1 of heptose moiety)]. After the cleavage of the isopropylidene group of 10 with 95% trifluoroacetic acid[12] at 0°C, hydrogenolytic removal of the benzyl and phenyl groups was achieved with palladium and Adams' platinum catalysts in MeOH solution to give the heptose-containing trisaccharide (1) [52%, mp 148-150℃ , $[\alpha]_D^{20}$ +4.3° (c=0.24, MeOH)] as the free acid form. In the positive fast atom bombardment mass spectrometry (FAB-MS), 1 revealed an $(M+H)^+$ ion at m/z 1449 and an $(M+Na)^+$ ion at m/z 1471. Compound 1 gave a positive test with the specific spray-reagent for phosphate.[13]



R= $-C_{14}OC_{14}$: $CH_3(CH_2)_{10}\overset{(R)}{C}HCH_2CO-$ CA : $ClCH_2CO-$, BOM : $PhCH_2OCH_2-$
$CH_3(CH_2)_{12}C(O)O$

Reagents: a) CA_2O, pyridine, DMAP, in CH_3CN r.t. 48h, b) $PhCHN_2$, in CH_2Cl_2 r.t. 30min
c) $TiBr_4$, in CH_2Cl_2-AcOEt (10:1) r.t. 24h, d) $Hg(CN)_2$, $HgBr_2$, in CH_2Cl_2 r.t. 48h
e) i) HDTC, in 2,6-lutidine-AcOH (3:1) 0°C 2h, ii) $CH_3C(OCH_3)=CH_2$, PTSA, in DMF r.t. 12h
f) BOM-Cl, pyridine, in CH_2Cl_2 r.t. 24h ; g) **9**, PTSA, in CH_2Cl_2 r.t. 24h
h) i) 95%CF_3COOH, in CH_2Cl_2 0°C 15min, ii) $Pd(OH)_2/H_2$, in MeOH r.t. 3h, iii) PtO_2/H_2, in MeOH r.t. 6h

Preliminaly examination of the biological activity showed that compound 1 has the same level of mitogenicity and lethal toxicity as that of the parent D-glucosamine-4-phosphate.

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11) Compound 9 was prepared from Benzyl 2,3,4,7-tetra-O-benzyl-β-L-glycero-D-manno-heptopyranoside[5a] in 4 steps, i) NaH, BzlBr/Bu_4NI/THF, 83% yield. ii) Ac_2O, H_2SO_4/AcOEt, 61% yield. iii) NH_4OH-MeOH (1:10), 90% yield. iv) Cl_3CCN, NaH/CH_2Cl_2, 73% yield.
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