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Synthesis of the Phosphodisaccharide Repeat of Antigenic Lipophosphoglycan of *Leishmania donovani* Parasite

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Abstract: Synthesis of the immunologically important and structurally unusual phosphodisaccharide repeat unit (Gal*p*1,4β-Man*p*-1α-phosphate) of the lipophosphoglycan cell surface GPI molecule of the protozoan parasite *Leishmania donovani* has been carried out using lactose as the starting material. The synthesis provides a short and stereoselective route for the preparation of this phosphosaccharide in a preparative scale.

Lipophosphoglycan (LPG) is the major glycoconjugate expressed on the cell surface of the promastigotes of the protozoan parasite *Leishmania donovani* which causes human visceral leishmaniasis (kala-azar) by infecting macrophages and subverting the host immune system (reviewed in ref. 1). The structure[2] of LPG consists of four distinct domains: (i) a 1-O-alkyl-2-*lyso*phosphatidyl(*myo*)inositol anchor; (ii) a conserved hexasaccharide core; (iii) a variable and highly negative charged repeating phosphodisaccharide of galactose and mannose residues; and (iv) a neutral mannose cap (Figure 1). The unique feature of the LPG structure is a phosphorylated disaccharide repeating sequence of [6Gal*p*-β1,4-Man*p*-α1-phosphate]$_n$ which provides a helical conformation.[3] The 1,4-β stereochemistry between Gal and Man is unique among eukaryotic carbohydrates. The number of the repeats is developmentally regulated[4] and multiplies during metacyclogenesis of the parasite. LPG is antigenic and a key virulence factor necessary for parasite infectivity and intracellular survival in macrophages by

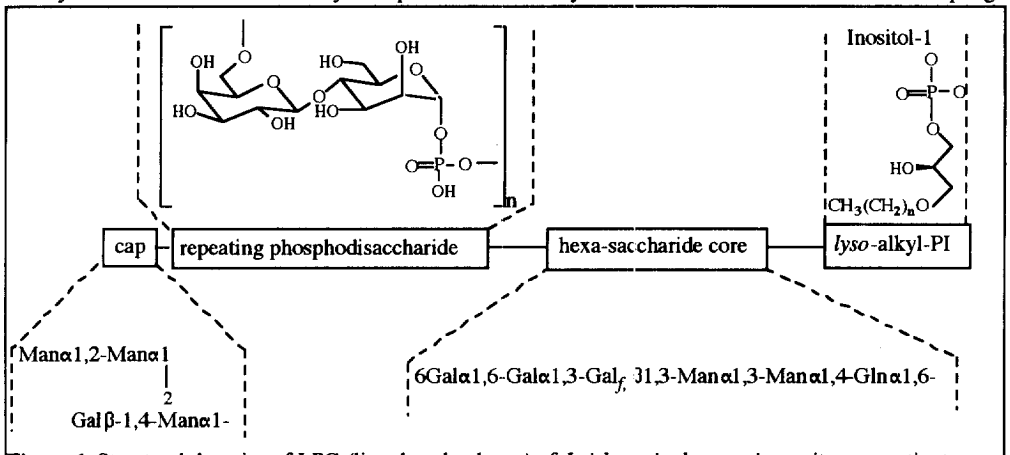

Figure 1. Structural domains of LPG (lipophosphoglycan) of *Leishmania donovani* parasite promastigotes (n = 16 to 32)

inhibition[5] of protein kinase C mediated signal transduction and related gene transcription.[6,7] The biosynthesis of the phosphodisaccharide repeat domain of LPG has been of considerable interest recently and a new enzyme α-D-mannosylphosphate-transferase has been identified[8] which utilises GDP-Man as the nucleotide sugar donor for transfer of mannose-1-α-phosphate to a Galβ1,4-Man-1-α-phosphate acceptor.[9] Immunological studies have shown the phosphosaccharide repeats as major epitopes presented[10] on the macrophage surface

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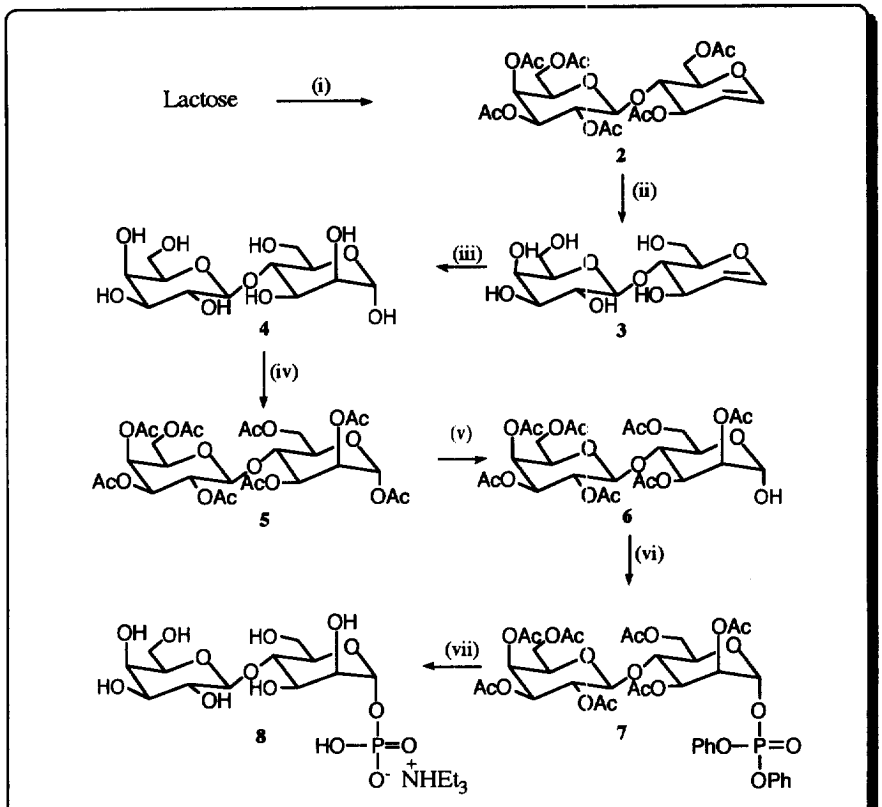
after processing of the parasitic antigenic molecules; monoclonal antibodies have also been raised recognising repeats. The LPG and its immunological role in host-parasite interaction has led to synthetic interest[11,12] and the assembly of phosphosaccharide repeat domains has been viewed as a target for development of chemotherapeutic agents, since this biosynthetic process also occurs in dividing intracellular amastigote forms of the parasite that propagate disease. Recently Nikolaev et. al.[12] reported a H-phosphonate chemistry based approach towards the phosphodisaccharide repeat and higher oligomers using the monosaccharide building blocks galactose and mannose.

In our ongoing work[13,14] on the chemistry of GPI related molecules of *Leishmania donovani*, we decided to explore the possibility of using synthetic phosphodisaccharide repeat and higher oligomers as antigenic molecules. Herein we report a new and efficient synthesis of the phosphodisaccharide repeat Gal*p*-β1,4-Man*p*-1-O-α-phosphate using readily available lactose as the starting material. The important features of this approach include, (a) a gluco-manno transformation *via* glycal chemistry to convert lactose (Gal-β1,4-Glu) into the intermediate disaccharide Gal-β1,4-Man; this avoided several protection/deprotection/glycosidation steps required in the synthesis from monosaccharide building blocks, (b) 1-O-deacylation and stereoselective phosphorylation to obtain the desired α-phosphate. The target phosphodisaccharide repeat **8** was synthesised from lactose as shown in Scheme 1.

The key intermediate hexa-O-acetyl lactal (**2**) was prepared from lactose by procedures used[15] in glycal chemistry (per-acetylation, anomeric bromination and Zn-AcOH mediated reductive elimination) in overall 58 % yield in three steps. The hexa-O-acetyl lactal, $[\alpha]_D$ -18 (c 1.0, $CHCl_3$)[15] was deacetylated using Na_2CO_3 in MeOH to give the lactal **3** (100 % yield, $[\alpha]_D$ +27 (c 1.6, H_2O)[15] which on treatment with *m*-chloroperoxy benzoic acid led to β-D-galactopyranosyl-(1-4)-α-D-mannopyranose (**4**)[15] in 90 % yield[16], and 10 % β-anomer. This was acetylated by acetic anhydride/pyridine to give 1,2,3,6-tetra-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-mannopyranose (**5**) as the major isomer which was purified by column chromatography, $[\alpha]_D$ +17.7 (c 0.9, $CHCl_3$); the 1-αH stereochemistry of the mannosyl residue was ascertained from NMR data (δ 6, *d*, *J* 1.9 Hz). The regioselective deacetylation of **5** at the anomeric position was carried out using saturated dimethylamine in acetonitrile at -20 ^{0}C to give 2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-mannose (**6**) in quantitative yield.[17] Stereoselective diphenylphosphorylation[18] (diphenylphosphorochloridate, BuLi, -78 ^{0}C, 20 min) led to 1-O-α-diphenylphosphate as the major isomer after column chromatography which was characterised as 2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-mannopyranosyl diphenyl phosphate (**7**, yield 47%). The α-stereochemistry of the diphenylphosphoryl group on the mannosyl residue was established by ^{1}H NMR which showed an anomeric proton, H-1, at δ 5.83 (*dd*, *J* 6.7, 1.9 Hz) with characteristic heteronuclear ^{1}H-^{31}P coupling of 6.7 Hz, and ^{31}P NMR at -13 ppm (for full spectral data see ref. 19). This was also confirmed by correlation of NMR data of **7** with those of 1-O-α-mannosylphosphate. The protected α-diphenylphosphate (**7**) was hydrogenated in a Parr hydrogenator at 55 psi using Adams' catalyst (PtO_2) to give the corresponding hepta-acetyl-phosphate which on further treatment with methanol-triethylamine-water (2:1:1) at 0 ^{0}C provided the desired β-D-galactopyranosyl-(1-4)-α-D-mannopyranosyl phosphate triethylammonium salt (**8**)[20] in 77 % yield. The H-1 signals of the α-D-mannosylphosphate (5.27, $J_{1H,P}$ 6.8 and $J_{1,2}$ 1.9 Hz) and β-D-galactopyranosyl (4.38, $J_{1',2'}$ 7.6 Hz) residues of **8** were unambiguously assigned by ^{1}H-^{1}H gradient-COSY, 2D-TOCSY (total correlation spectroscopy) and ^{31}P NMR experiments. The α-configuration of the mannosyl phosphate fragment was further confirmed from the chemical shift positions of C-3 (70.07 ppm) and C-5 (73.67 ppm) signals of **8**; the latter values were close to that of the C-3 and C-5 signals of α-D-mannopyranosyl phosphate[18] taking into account the influence



Scheme-1

Reagents and conditions: *(i) a. Ac_2O, NaOAc, 100 ^{0}C; b. HBr-AcOH, 18 ^{0}C, 2 hr; c. Zn-AcOH, $CuSO_4$, -5 ^{0}C. (ii) Na_2CO_3, MeOH, r.t., 2 hr. (iii) MCPBA, diethyl-ether-H_2O, r.t.(iv) Ac_2O, Py (v) Me_2NH-MeCN, -20 ^{0}C, 50 min. (vi) BuLi, -78 ^{0}C, $(PhO)_2POCl$. (vii) a. PtO_2, H_2, 50 psi, 16 hr; b. Et_3N, MeOH, H_2O, 0 ^{0}C.*

of the β-D-galactopyranosyl substituent at the 4 position.

Considering that Gal*p*-β1,4Man*p*-1-O-α-phosphate is immunologically important and serves as a biosynthetic substrate for the unique parasitic enzyme GDP-Man:Galβ1,4Manα-1phosphate:α-mannosyl phosphate-transferase, the synthesis presented here will enable design of synthetic immunogenic and antiparasitic enzyme inhibitors.

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16. Spectral data of compound **4**: $[\alpha]_D$ = +27 (c 1.5, H_2O)[15] ; ^1H NMR 300 MHz (D_2O) 5.12 (1H, *d*, J = 1.67, H-1 of α-anomer), 4.85 (*d*, J = 0.98, H-1 of minor β anomer), 4.40-4.36 (2H, *m*, H-1' and H-4), 3.75 (1H, *dd*, H-2'), 3.94-3.92 (2H, *m*, H-4' and H-2), 3.89-3.83 (2H, *m*, H-6'), 3.81-3.79 (1H, *dd*, $J_{3,4}$ = 6, $J_{3,2}$ = 2, H-3), 3.75-3.71 (2H, *m*, H-6), 3.63-3.59 (1H, *br dd*, H-3'), 3.51-3.46 (2H, *m*, H-5, H-5'); MS (ES^-) 341 $[M-H]^-$; HRMS (FAB^+):m/z 365.106559 $[M+Na]^+$ ($C_{12}H_{22}O_{11}Na$ requires 365.105982).
17. Spectral data of compound **6**: $[\alpha]_D$ = +20 (c 0.63, $CHCl_3$); ^1H NMR ($CDCl_3$) 5.45 (1H, *br d*, H-4'), 5.42 (1H, *d*, $J_{3,2}$ = 3, H-3), 5.38 (1H, *dd*, H-2), 5.22 (1H, *dd*, $J_{1,2}$ = 1.5, $J_{1,OH}$ = 3.0, H-1), 5.11 (1H, *m*, H-2'), 4.95 (1H, *dd*, $J_{3',4'}$ = 3.4, H-3'), 4.53 (1H, *d*, $J_{1',2'}$ = 7.9, H-1'), 4.43 (1H, *dd*, $J_{4,5} = J_{3,4}$ = 9.69, H-4), 4.2-4.0 (2H, *m*, H-6), 4.15 (1H, *m*, H-5), 3.91 (1H, *br dd*, H-5'), 3.80-3.90 (2H, *m*, H-6'), 1.8-2.2 (21H, 7 x s, COMe); MS (ES^+), 636 $[M]^+$; HRMS (FAB^+):m/z 659.178990 $[M+Na]^+$ ($C_{26}H_{36}O_{18}Na$ requires 659.179935).
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19. Spectral data of compound **7**: $[\alpha]_D$ = +1.93 (c 0.46, $CHCl_3$); ^1H NMR ($CDCl_3$, assignments confirmed by 2D COSY and TOCSY experiments) 7.36-7.26 (10H, *m*, 2 x Ph), 5.83 (1H, *dd*, J_{1H-P} = 6.7, $J_{1,2}$ = 1.9, H-1), 5.40 (1H, *m*, H-3), 5.35 (1H, *br d*, H-4') 5.30 (1H, *m*, H-2), 5.13 (1H, *m*, H-2'), 4.96 (1H, *dd*, $J_{3',4'}$ = 3.4, H-3'), 4.54 (1H, *d*, $J_{1',2'}$ = 7.89, H-1'), 4.47 (1H, *d*, $J_{4,5}$ = 7.81, H-4), 4.24-4.13 (2H, *m*, H-6), 4.15 (1H, *m*, H-5), 3.80-3.95 (2H, *m*, H-6'), 3.96 (2H, *m*, H-5'), 1.8-2.2 (21H, 7 x s, COMe) ; ^{31}P NMR, -13.0 (external reference H_3PO_4); MS (ES^+), 891 $[M+Na]^+$; HRMS (FAB^+):m/z 891.217085 $[M+Na]^+$ ($C_{38}H_{45}O_{21}NaP$ requires 891.208867).
20. Spectral data of compound **8**: $[\alpha]_D$ = +10 (c 0.1, H_2O); ^1H NMR (D_2O, assignments confirmed by 2D COSY and TOCSY experiments, 5.27 (1H, *dd*, J_{1H-P} = 6.8, $J_{1,2}$ = 1.9, H-1), 4.38 (1H, *d*, $J_{1',2'}$ = 7.6, H-1'), 4.38 (1H, *d*, $J_{4,5}$ = 9.65, H-4),, 3.94 (1H, *m*, H-2), 3.83 (1H, *m*, H-4'), 3.83 (2H, *m*, H-6'), 3.76 (1H, *t*, $J_{3,4}$ = 7.11, $J_{3,2}$= 2.64, H-3), 3.68 (2H, *m*, H-6), 3.56-3.53 (2H, *m*, H-2, H-3'), 3.60 (1H, *m*, H-5'), 3.46 (1H, m, H-5), 3.45 (1H, *dd*, $J_{1',2'}$ = 6.67, $J_{2',3'}$ = 1.5, H-2') ; ^{31}P NMR, -2.07 (external reference H_3PO_4); HRMS (ES^-): 421.27185 $[M-1H]^-$ ($C_{12}H_{22}O_{14}P$ requires 421.27200).