

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

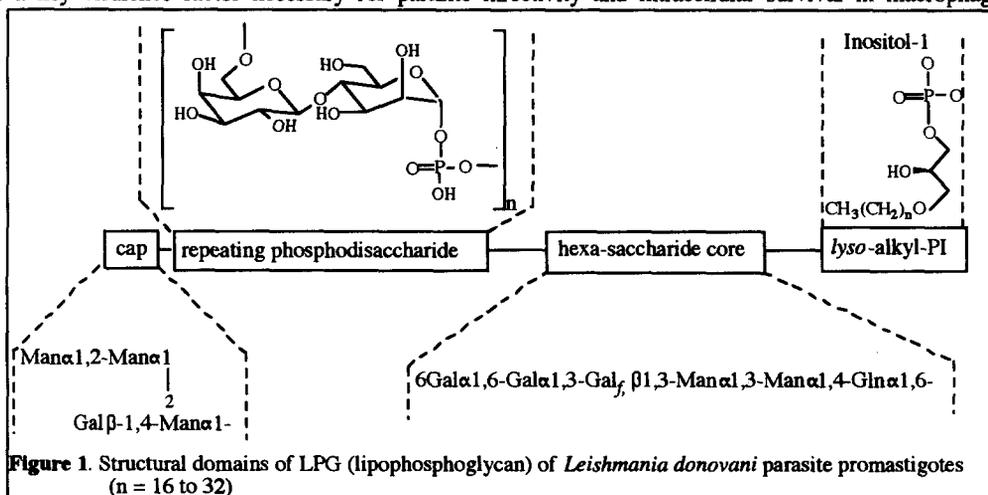
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Abstract: Synthesis of the immunologically important and structurally unusual phosphodisaccharide repeat unit (Gal β 1,4-Man α 1-phosphate) of the lipophosphoglycan cell surface GPI molecule of the protozoan parasite *Leishmania donovani* has been 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.
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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 structure² of LPG consists of four distinct domains: (i) a 1-O-alkyl-2-lysophosphatidyl(*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 β -1,4-Man α -1-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 regulated⁴ 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

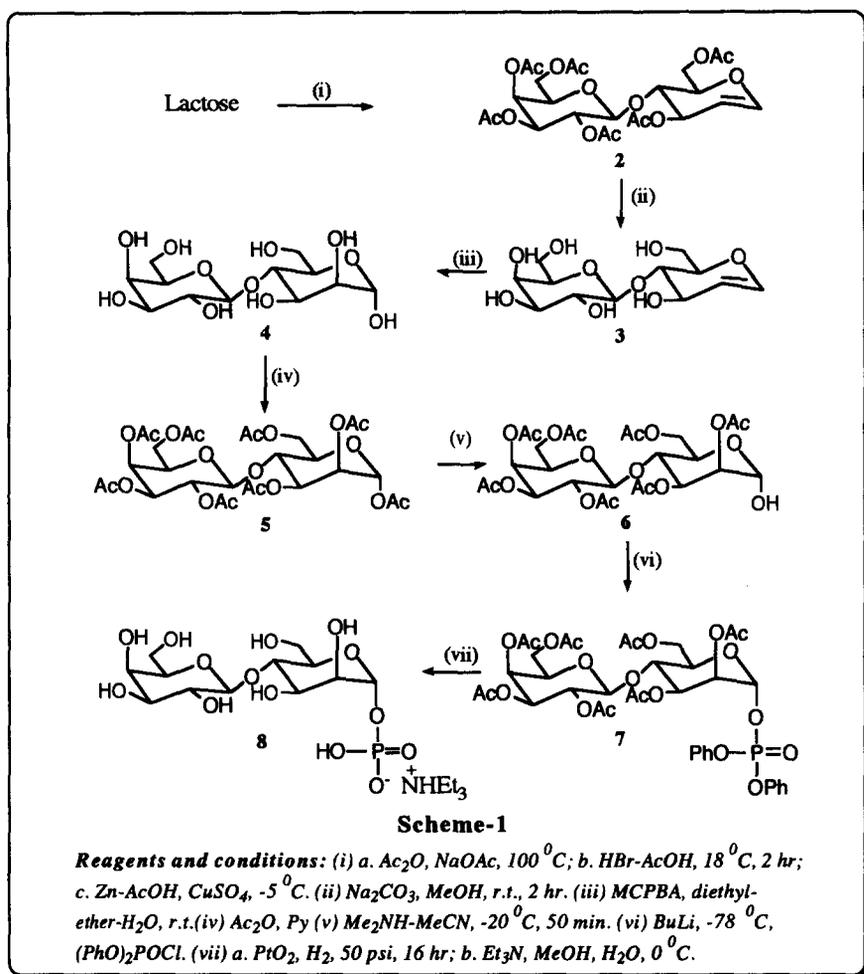


inhibition⁵ of protein kinase C mediated signal transduction and related gene transcription.^{6,7} The biosynthesis of the phosphodisaccharide repeat domain of LPG has been of considerable interest recently and a new enzyme α -D-mannosylphosphate-transferase has been identified⁸ which utilises GDP-Man as the nucleotide sugar donor for transfer of mannose-1- α -phosphate to a Gal β 1,4-Man-1- α -phosphate acceptor.⁹ Immunological studies have shown the phosphosaccharide repeats as major epitopes presented¹⁰ on the macrophage surface

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 Galp- β 1,4-Manp-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- β 1,4-Glu) into the intermediate disaccharide Gal- β 1,4-Man; this avoided several protection/deprotection/glycosidation steps required in the synthesis from monosaccharide building blocks, (b) 1-O-deacetylation 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 used¹⁵ 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, CHCl₃)¹⁵ was deacetylated using Na₂CO₃ in MeOH to give the lactal **3** (100 % yield, $[\alpha]_D +27$ (c 1.6, H₂O)¹⁵ which on treatment with *m*-chloroperoxy benzoic acid led to β -D-galactopyranosyl-(1-4)- α -D-mannopyranose (**4**)¹⁵ in 90 % yield¹⁶, 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, CHCl₃); the 1- α H 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 °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 diphenylphosphorylation¹⁸ (diphenylphosphorochloridate, BuLi, -78 °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 ¹H NMR which showed an anomeric proton, H-1, at δ 5.83 (*dd*, *J* 6.7, 1.9 Hz) with characteristic heteronuclear ¹H-³¹P coupling of 6.7 Hz, and ³¹P 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 (Pt₂O) to give the corresponding hepta-acetyl-phosphate which on further treatment with methanol-triethylamine-water (2:1:1) at 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 ¹H-¹H gradient-COSY, 2D-TOCSY (total correlation spectroscopy) and ³¹P 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 phosphate¹⁸ taking into account the influence



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

Considering that $\text{Galp-}\beta\text{1,4Manp-1-O-}\alpha\text{-phosphate}$ is immunologically important and serves as a biosynthetic substrate for the unique parasitic enzyme $\text{GDP-Man:Gal}\beta\text{1,4Man}\alpha\text{-1phosphate:}\alpha\text{-mannosyl phosphate-transferase}$, 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, H₂O)¹⁵; ¹H NMR 300 MHz (D₂O) 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]⁺ (C₁₂H₂₂O₁₁Na requires 365.105982).
17. Spectral data of compound 6: $[\alpha]_D = +20$ (c 0.63, CHCl₃); ¹H NMR (CDCl₃) 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]⁺ (C₂₆H₃₆O₁₈Na requires 659.179935).
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19. Spectral data of compound 7: $[\alpha]_D = +1.93$ (c 0.46, CHCl₃); ¹H NMR (CDCl₃, 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); ³¹P NMR, -13.0 (external reference H₃PO₄); MS (ES⁺), 891 [M+Na]⁺; HRMS (FAB⁺): m/z 891.217085 [M+Na]⁺ (C₃₈H₄₅O₂₁NaP requires 891.208867).
20. Spectral data of compound 8: $[\alpha]_D = +10$ (c 0.1, H₂O); ¹H NMR (D₂O, 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'); ³¹P NMR, -2.07 (external reference H₃PO₄); HRMS (ES⁻): 421.27185 [M-1H]⁻ (C₁₂H₂₂O₁₄P requires 421.27200).