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# A facile and novel route to the antigenic branched phosphoglycan of the protozoan *Leishmania major* parasite ☆

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**Abstract**—A novel approach for the iterative synthesis of the antigenic branched phosphoglycan of the protozoan parasite *Leishmania major* is presented that exploits remarkable dual selectivity in the functionalization of D-lactal, providing flexibility to extend the PG chain either towards the reducing or nonreducing end.


The protozoan parasite *Leishmania*, responsible for multiple diseases ranging from cutaneous to fatal visceral leishmaniasis, survives in extremely hostile milieus throughout its life cycle in the sand-fly vector and the human host. For this, all *Leishmania* species synthesize[1] a unique class of molecules termed phosphoglycans, including the membrane-bound lipophosphoglycan (LPG) expressed by the infectious promastigote form, and the proteophosphoglycan (PPG) secreted by the intracellular amastigote form of the parasite. Their role(s) in parasite virulence has been a topic of intense debate[2] in recent years but now it has been established[3] that the principal virulent determinant in *Leishmania major* consists of the PG repeat units, independent of their molecular platform (lipid linked in LPG and peptide linked in PPG). The intriguing structure[4] of LPG (Fig. 1) consists of four distinct functional domains; the alkyllysophosphatidylinositol lipid anchor, the conserved glycan core with an internal $Gal_f$ residue, variable phosphoglycan (PG) repeats, and a neutral oligomannose cap. The most distinct feature of LPG is the dynamic PG domain made of [-6Gal*p*-β1,4-Man*p*-α1-phosphate]$_n$ repeats ($n = 2$–32), which is unique among all the eukaryotic carbohydrates. Although LPG is expressed by all *Leishmania* species, there is one remarkable difference between the structure of LPG from *L. major* and that from other species; the 3-OH position of the galactose residue of PG repeats is highly substituted with 1-3β-galactosyl branches in *L. major* (as shown in Fig. 1), whereas this position remain totally unsubstituted in *Leishmania donovani* where the PGs are linear.

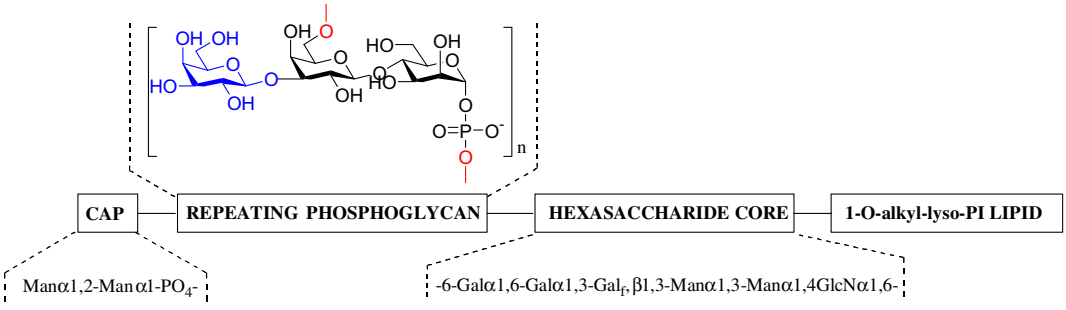


**Figure 1.** Structure of lipophosphoglycan of *L. major*.

☆ Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tetlet.2004.01.155

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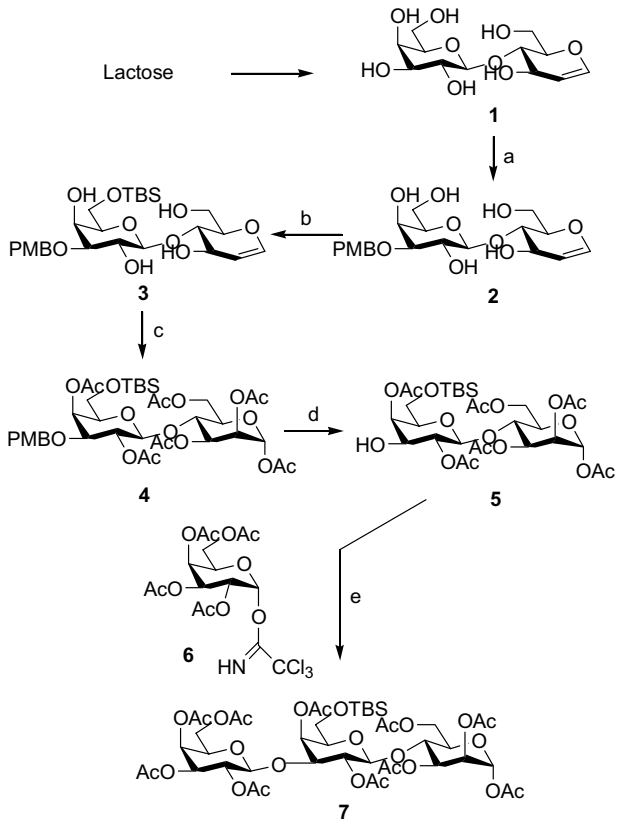
These remarkable inter-species structural differences are clearly manifested[5] biologically, for example, in *L. major*, the branched PG 6-[Gal(β1→3)]Gal*p*-β1,4-Man*p*-α1-phosphate controls[5] the transformation of the noninfective procyclic form of the parasite to the infectious metacyclic form, and also constitutes[6] the major epitope for the attachment of the virulent promastigotes to the sand-fly mid gut and human macrophages.

The unique structure and function of LPG have generated significant interest[1,7] as a target for new chemo- and immunotherapy, but to exploit this knowledge requires efficient synthetic approaches for the construction of phosphoglycans of defined lengths and repeats and their *neo*-glycoconjugates. The synthesis of the repeating PGs is complicated, as compared to that of nonanomeric types such as oligonucleotides, due to presence of anomeric phosphodiesters between each repeat unit, which places additional requirements[8,9] of anomeric stereocontrol during the synthesis, and also leads to instability due to the propensity of the glycosyl ring to form a stabilized carbocation by expulsion of the anomeric phospho monoester leaving group. The branched *L. major* phosphoglycans present specific problems due to the presence of 3′-galactosyl branches, and only one approach to the branched PG of *L. major* has been reported[10,11] so far that starts from monosaccharide building blocks using multiple protection, glycosylation and phosphorylation steps and the synthesis is extendable only towards the nonreducing end.

In continuation of our efforts on the chemistry[12–15] and biology[16] of LPG, we now report a facile and novel route for the rapid construction of the branched phosphoglycan of *L. major*, with the flexibility to extend the PG repeats on either the nonreducing or reducing ends. Key features of our approach include (a) remarkable dual selectivity in protection of the easily available starting material, D-lactal, with mutually compatible groups at strategic positions, (b) a *gluco–manno* transformation followed by 3′-galactosylation to give the central trisaccharide intermediate ready to serve either as the phosphate donor or acceptor for the rapid assembly of the phosphohexasaccharide. The starting material D-lactal **1** is easily prepared from lactose in four straight forward steps (acetylation, bromination, reductive elimination and deacetylation). The next step (Scheme 1) was to selectively protect the 3′-OH and 6′-OH positions of the galactose residue with two mutually compatible groups. This was achieved by first placing a *p*-methoxybenzyl (PMB) group on the 3′-OH of **1** by dibutyltin chemistry ($Bu_2SnO$, MeOH, reflux; PMBCl, CsF, KI), which led exclusively to the 3′-*O*-PMB-D-lactal **2**; the position of the PMB group was confirmed[17] by an HMBC experiment showing a correlation between the 3′-H ($\delta$ 3.53 ppm) and the benzylic carbon (70.72) of the PMB group. The next critical step that is, selective protection of the 6′-OH, was achieved by reaction of **2** with an equimolar amount of *tert*-butyldimethylsilyl chloride (TBSCl) using NaH, and catalytic amounts of the crown ether (18-crown-6). This NaH/crown-ether mediated silylation showed remarkable regioselectivity

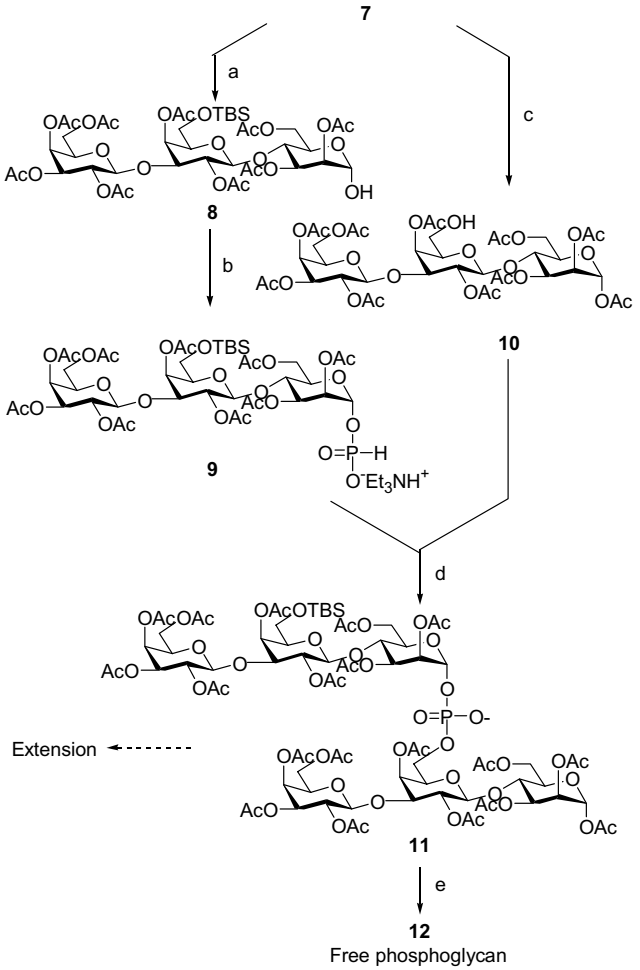



**Scheme 1.** Reagents and conditions: (a) $Bu_2SnO$, MeOH, reflux, 4 h; PMBCl, CsF, KI, DMF, rt, 48 h, 74%; (b) NaH, 18-crown-6, TBSCl, THF, 0 °C–rt, 30 min, 65%; (c) (i) *m*-CPBA, ether–$NaHCO_3$ buffer, 0 °C, 3 h, (ii) $Ac_2O$, py, rt, 16 h, 80%; (d) DDQ, $CH_2Cl_2$–$H_2O$, rt, 12 h, 85%; (e) compounds **5** and **6**, ether, TMSOTf, −20 °C, 4 h, 74%.

in favor of the 6-OH of the Gal residue over the 6-OH of the glucal residue (product ratio 85:15) providing the desired 3′-*O*-PMB-6′-*O*-TBS-D-lactal **3** in good yield. The placement of TBS and PMB groups was assigned by $^1H$–$^{13}C$ HMBC experiments and the 6′-position of the TBS was further confirmed[18] by a silyl-induced shift of C-6′ (61.21) as compared to C-6 (62.45). The selectivity of silylation under the above conditions was observed only when the PMB group was present at the 3′-position, indicating that the PMB group selectively enhanced the nucleophilicity of the proximal $CH_2OH$ group of the galactose residue. The reverse sequence, selective silylation at 6′-OH followed by alkylation at 3′-OH, failed and yielded intractable mixtures. The next two steps involved the stereoselective *gluco* → *manno* transformation of **3** by a sodium bicarbonate catalyzed *m*-CPBA reaction under biphasic (5% aq bicarbonate-diethyl ether) conditions providing, in high yield, the *manno* compound 3′-*O*-PMB-6′-*O*-TBS-Gal-(β1→4)-Man, which on direct per-acetylation led to the key intermediate 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,4-di-*O*-acetyl-3-*O*-PMB-6-*O*-TBS-β-D-galactopyranosyl)-α-D-mannopyranose **4**[19] as the major isomer. Now the PMB group from compound **4** was removed by DDQ in acetonitrile to obtain the disaccharide acceptor **5** in a 85% yield, which was then coupled (DCM, TMSOTf at −20 °C) with the freshly prepared[20] 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-trichloroacetimidate glycosyl donor **6**.

This galactosylation reaction turned out to be efficient and provided the desired major β-isomer,[21] 1,2,3,6-tetra-*O*-acetyl-4-*O*-[6-*O*-TBS-2,4-di-*O*-acetyl-3*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-galactopyranosyl]-α-D-mannopyranose **7**.

The suitably protected trisaccharide **7** was ideally placed to serve as the central point to access both the *H*-phosphonate donor **9** as well as the PG-acceptor **10** (Scheme 2) for rapid and iterative synthesis of the target branched phosphoglycan **11**. For this, compound **7** was divided into two parts; the first part was converted into the PG donor by (a) anomeric deacetylation by freshly prepared saturated dimethylamine in acetonitrile at −20 °C to provide compound **8** in quantitative yield followed by (b) phosphitylation using triimidazolyl phosphine generated in situ from $PCl_3$ and imidazole. These two high yielding steps led to 2,3,6-tri-*O*-acetyl-4-*O*-[2,4-di-*O*-acetyl-6-*O*-TBS-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-galactopyranosyl]-α-D-mannopyranosyl *H*-phosphonate **9**, which was isolated as its triethyl ammonium bicarbonate salt. The $^1H$ and $^{31}P$ NMR of **9** clearly showed[22] characteristic signals for its anomeric *H*-phosphonate group [($\delta_P$ − 0.13; $\delta_H$ 5.48 (dd, $J_{1,2}$ 1.9, $J_{1,P}$ 8.0, H-1), 6.90 (d, $J_{H,P}$ 642, H–P)]. The second part of the compound **7** was converted to the PG-acceptor **10** by HF mediated (aq HF–$CH_3CN$, 5:95, 0 °C, 2 h) removal of TBS from the 6-position of the distal galactose residue.




**Scheme 2.** (a) $Me_2NH$, $CH_3CN$, −20 °C, 3 h, 95%; (b) $PCl_3$, imidazole, $CH_3CN$, 0 °C, 2 h, TEAB work-up, 87%; (c) HF–$CH_3CN$, 0 °C, 2 h, 85%; (d) compounds **9** and **10**, pivaloyl-Cl, pyridine, rt, 1 h; iodine in aq py, 0.5 h, TEAB work-up, 79%; (e) 48% HF–$CH_3CN$, 5:95, 0 °C, 2 h; NaOMe–MeOH, 95%.

Having secured efficient access to both the trisaccharide *H*-phosphonate donor **9** and the trisaccharide acceptor **10**, their coupling was carried out using pivaloyl chloride followed by in situ iodine oxidation to give the fully protected phosphohexasaccharide **11**.[23] The presence of (1 → 6)-phosphodiester linkages between the PG repeats was confirmed by $^{31}P$–$^{13}C$ coupling (doublets, 5 and 7 Hz in $^{13}C$ NMR) for C-1 and C-2 of the Man units, and C-5 and C-6 of the corresponding Gal units; these $^{13}C$ signals were shifted due to the α- and β-phosphorylation effect. The α-configuration of the mannosyl phosphate linkage was confirmed by an $^1H$ NMR analysis that showed characteristic coupling (5.45 ppm, $J_{H,H} = 1.9$ and $J_{H,P} = 7$ Hz) for the corresponding anomeric proton. This was further confirmed from the $^{13}C$ signals of the mannose C-3 and C-5 positions, corresponding to those reported[24,25] for α-mannosyl phosphate containing phosphoglycans. The protected phosphohexasaccharide **11**, on global deprotection with HF–$CH_3CN$ and NaOMe–$CH_3OH$ conditions, provided free phosphoglycan **12** (with two branched PG repeats).

Evidently the protected phosphohexasaccharide **11** is fairly well placed for further iterative extension of the phosphoglycan chain either towards the upstream non-reducing the 6′-OH end (removal of the TBS group followed by coupling with *H*-phosphonate donor **9**), or downstream reducing the 1-OH end (anomeric-deacetylation, phosphitylation and coupling with the trisaccharide acceptor **10**) following the same methods as described above. Furthermore, this synthetic approach is eminently suitable for adaptation on the solid-phase mode for preparation of larger branched phosphoglycans of *L. major* by the application of our recently described[15] *cis*-allyloxyphosphoryl linker strategy.

In conclusion, a versatile synthesis for the rapid assembly of the branched phosphoglycans, antigenic virulence factors of *L. major*, has been designed that allows the PG chain to grow in either direction. We are now extending this approach on solid phase for the preparation of larger phosphoglycans, and their conjugation with suitable carrier antigenic proteins such as KLH to test such *neo*-glycoconjugates as potential vaccines against *L. major* infection. Full details of the solution and solid phase synthesis and immunological results will be reported in due course.

## Supplementary material

Experimental and characterization data for all new compound **2**–**5** and **7**–**12**.

## Acknowledgements

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17. Characterization data for **2**: $^1$H NMR (acetone-$d_6$) $\delta$ 3.53 (dd, $J = 3.3$, 9.6 Hz, 1H), 3.71–3.79 (m, 10H), 3.80–4.00 (m, 6H), 4.15 (d, 1H), 4.32 (d, 1H), 4.54 (d, $J = 7.8$ Hz, 1H), 4.63–4.77 (m, 3H), 6.28 (d, $J = 6.2$ Hz, 1H), 6.86 (d, $J = 8.4$ Hz, 2H), 7.33 (d, $J = 8.4$ Hz, 2H); $^{13}$C NMR (acetone-$d_6$) $\delta$ 54.36, 61.25, 65.80, 68.24, 70.35, 70.72, 75.52, 77.20, 80.91, 102.96, 104.39, 113.42, 129.26, 143.23; HRMS (ESMS+): $m/z$ 451.1420 $(M+Na)^+$ ($C_{20}H_{28}O_{10}Na$ requires 451.1580).
18. Characterization data for **3**:$^1$H NMR (acetone-$d_6$) $\delta$ 0.07 (s, 6H), 0.89 (s, 9H), 3.49–3.58 (m, 2H), 3.60–3.68 (m, 3H), 3.72–3.87 (m, 11H), 4.07 (d, 1H), 4.23 (d, 1H), 4.46 (d, $J = 7.8$ Hz, 1H), 4.57–4.69 (m, 3H), 6.27 (d, $J = 6.2$ Hz, 1H), 6.87 (d, $J = 8.4$ Hz, 2H), 7.33 (d, $J = 8.4$ Hz, 2H); $^{13}$C NMR (acetone-$d_6$) $\delta$ −6.32 to −6.23, 17.94, 25.36, 54.51, 61.21, 62.45, 65.32, 68.16, 70.30, 70.83, 75.55, 77.12, 81.07, 81.50, 103.04, 104.57, 113.38, 129.18, 143.08; HRMS (ESMS+): $m/z$ 565.2430 $(M+Na)^+$ ($C_{26}H_{42}O_{10}SiNa$ requires 565.2445).
19. Selected data for **4**:$^1$H NMR ($CDCl_3$) $\delta$ 0.02 (s, 6H), 0.87 (s, 9H), 1.97–2.11 (m, 18H), 3.42–3.88 (m, 4H), 4.08–4.13 (m, 2H), 4.28–4.43 (m, 3H), 4.56–4.63 (m, 2H), 4.94–5.01 (m, 3H), 5.19 (br d, 1H), 5.32 (dd, $J = 4.8$, 8.1 Hz, 1H), 5.55 (br d, 1H), 5.98 (d, $J = 1.8$ Hz, 1H), 6.83 (d, $J = 8.4$ Hz, 2H), 7.21 (d, $J = 8.4$ Hz, 2H); $^{13}$C NMR ($CDCl_3$) $\delta$ −5.77, 18.07, 20.46–20.73, 25.65, 55.15, 60.41, 68.24, 69.39, 70.77, 70.87, 71.01, 73.49, 73.74, 76.38, 90.28, 101.29, 113.70, 129.34–130.01, 159.26–170.39; HRMS (ESMS+): $m/z$ 851.3015 $(M+Na)^+$ ($C_{38}H_{56}O_{18}SiNa$ requires 851.3134).
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21. Selected data for **7**:$^1$H NMR ($CDCl_3$) $\delta$ 0.01 (s, 6H), 0.83 (s, 9H), 2.07–2.16 (m, 30H, 10×OAc), 3.50 (m, 4H), 4.08–4.15 (m, 6H), 4.25–4.40 (m, 3H), 4.56–4.63 (m, 4H), 4.94–5.10 (m, 3H), 5.20 (m, 2H), 5.32 (dd, $J = 4.6$, 8.1 Hz, 1H), 6.29 (d, $J = 1.8$ Hz, 1H); $^{13}$C NMR $\delta$ −5.77, 13.99, 20.42–20.85 (10×OCO*C*H$_3$), 60.38, 60.89, 61.17, 66.60, 67.43, 68.03, 68.35, 69.15, 70.30, 70.97, 72.36, 73.08, 73.67, 77.11, 91.87, 101.02, 101.45, 168.15–170.65 (10×O*CO*CH$_3$); HRMS (ESMS+): $m/z$ 1061.3512 $(M+Na)^+$ ($C_{44}H_{66}O_{26}SiNa$ requires 1061.3509).
22. Selected data for **9**:$^1$H NMR ($CDCl_3$) $\delta$ 0.05 (s, 6H), 0.86 (s, 9H), 1.30 (t, 9H, TEA ion), 2.04–2.14 (m, 27H, 9×OAc), 3.10 (q, 6H, TEA ion), 3.50 (m, 4H), 4.08–4.15 (m, 6H), 4.25–4.40 (m, 3H), 4.56–4.63 (m, 4H), 4.93–5.20 (m, 4H), 5.20 (m, 2H), 5.32 (dd, $J = 4.7$, 8.1 Hz, 1H), 5.48 (dd, $J = 1.9$, 8.0 Hz, 1H), 6.90 (d, $J_{H,P} = 642$ Hz, 1H); $^{13}$C NMR $\delta$ −5.70, 13.90, 20.45–20.80 (9×OCO*C*H$_3$), 60.38, 60.89, 61.17, 66.60, 67.43, 68.03, 68.35, 69.15, 70.30, 70.97, 72.36, 73.08, 73.67, 77.11, 92.54, 101.2, 101.5, 168.0–169.23 (9×O*CO*CH$_3$); $^{31}$P NMR: $\delta = 0.13$; HRMS (ESMS−): $m/z$ 1059.340 $(M-H)^-$ ($C_{42}H_{65}O_{27}SiP$ requires 1059.3142).
23. Selected data for **11**: $^1$H NMR ($CDCl_3$) $\delta$ 0.01 (s, 6H), 0.84 (s, 9H), 1.30 (t, 9H), 1.96–2.15 (m, 57H), 3.00 (q, 6H),3.50 (m, 6H), 3.87 (m, 2H), 3.94 (m, 2H), 4.07–4.14 (m, 2H), 4.35 (m, 1H), 4.39 (m, 4H), 4.40 (m, 1H), 4.48 (m, 1H), 4.52 (m, 1H), 4.94 (d, $J = 7.7$ Hz, 3H), 5.28 (m, 5H), 5.43 (m, 1H), 5.45 (dd, $J_{H,H} = 1.9$ and $J_{H,P} = 7.0$ Hz, 1H), 5.46 (m, 1H), 6.01 (d, $J = 2.7$ Hz, 1H); $^{13}$C NMR: −5.75, 17.95, 25.57, 8.5, 45.50 (TEA ion), 20.48–20.79 (19 peaks), 60.06, 60.42 (d, $J_{C,P} = 8$ Hz), 62.22, 62.63, 66.55, 67.46, 68.27, 68.64, 69.37, 69.66, 69.84, 70.14, 70.75, 70.88, 71.20, 73.31, 73.76, 74.24, 77.15, 78.95, 90.41, 91.69, 101.08, 101.29, 168; $^{31}$P NMR: $\delta$ −2.90 (dt, $J_{P,H} = 7.5$, 10 Hz); HRMS (ESMS−): $m/z$ 1981.5725 $(M-H)^-$ ($C_{80}H_{115}O_{53}PSi$ requires 1981.5732).
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