Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 6, pp. 785-788, 1994

0960-894X/94 $6.00+0.00

0960-894X(94)E0051-F

The Chemical Synthesis of *Leishmania donovani* Phosphoglycan Fragments

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Abstract: The tetraglycosyl monophosphate **1**, hexaglycosyl diphosphate **2** and octaglycosyl triphosphate **3**, which are fragments of the phosphoglycan portion of *Leishmania donovani* lipophosphoglycan, have been prepared using disaccharide H-phosphonates for construction of the phosphodiester linkages.

The lipophosphoglycan (LPG) produced by the promastigote stage of the protozoan parasite *Leishmania donovani* is the most abundant macromolecule on the cell surface. The phosphoglycan portion is a linear poly(glycosyl phosphate) consisting of β-D-galactosyl-(1-4)-α-D-mannosyl phosphate repeat units and capped by a D-mannobiosyl phosphate unit at the nonreducing end[1]. We now report the synthesis of the tetra-, hexa- and octa-saccharide fragments **1-3** of the phosphoglycan. All the synthetic oligomers contain a 9-decenyl aglycone moiety and are designed to be used for both biosynthetic studies and the preparation of artificial antigens.

Man*p*(α1-2)Man*p*(α)-PO_4H-[6Gal*p*(β1-4)Man*p*(α)-PO_4H]$_{16}$ -- glycosyl phosphatidylinositol anchor

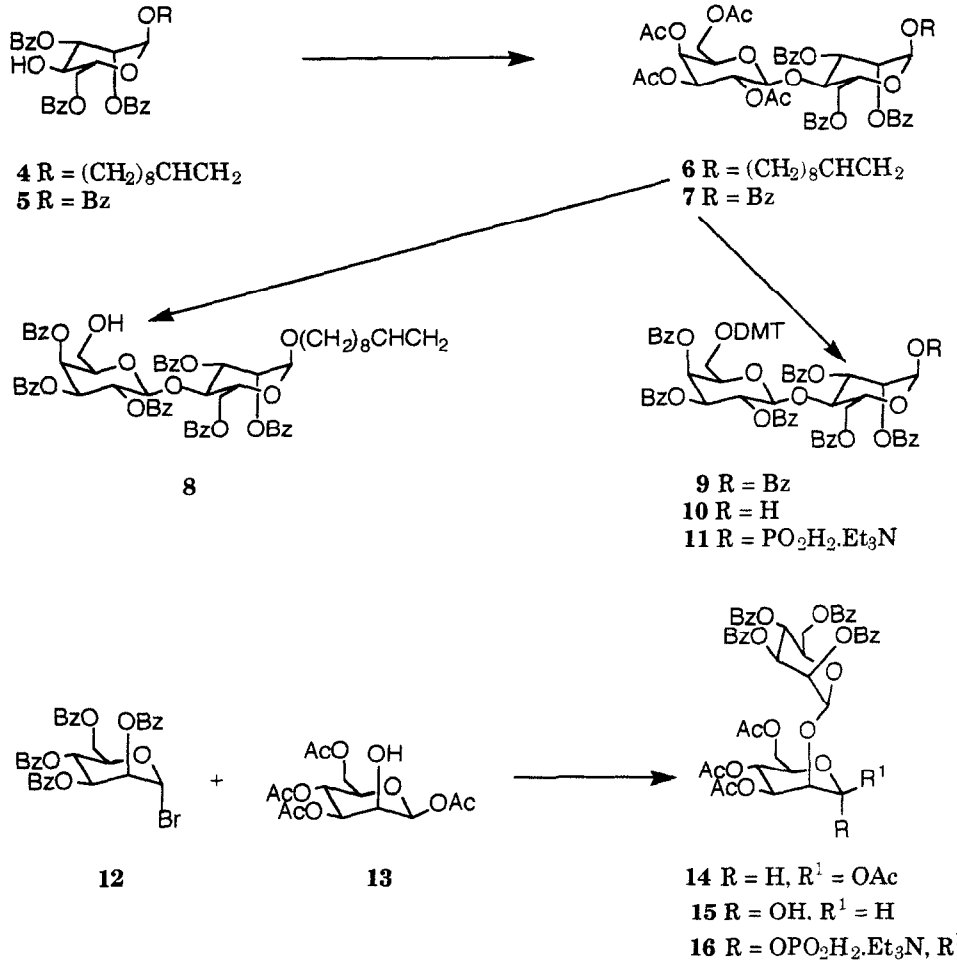
LPG *Leishmania donovani*

Gal*p*(β1-4)Man*p*(α)-[PO_4H-6Gal*p*(β1-4)Man*p*(α)]$_n$-O$(CH_2)_8CHCH_2$ **1** n = 1, **2** n = 2

Man*p*(α1-2)Man*p*(α)-[PO_4H-6Gal*p*(β1-4)Man*p*(α)]$_3$-O$(CH_2)_8CHCH_2$ **3**

Our approach is based on the use of the glycosyl H-phosphonate method[2] for the synthesis of the phosphodiester linkages, with the glycobiosyl H-phosphonate derivatives **11** and **16** being used for stepwise elongation of the oligomeric chain. The monohydroxylic 9-decenyl bioside **8** served as the first acceptor during the elongation. It was prepared using acetobromogalactose and the 2,3,6-tri-O-benzoyl-α-D-mannoside **4** (ref. 3) as the starting materials. The base deficient glycosylation reaction ($AgSO_3CF_3$ and 2,4,6-collidine)[4] resulted in β-linked disacharide **6** (67%) and its α-linked isomer (15%). Derivative **8** (ref. 5) was prepared by O-deacetylation of **6** with HCl/MeOH[6], followed by successive treatment with dimethoxytrityl chloride and benzoyl chloride in pyridine and detritylation (TFA/CH_2Cl_2) in an overall yield of 71% .

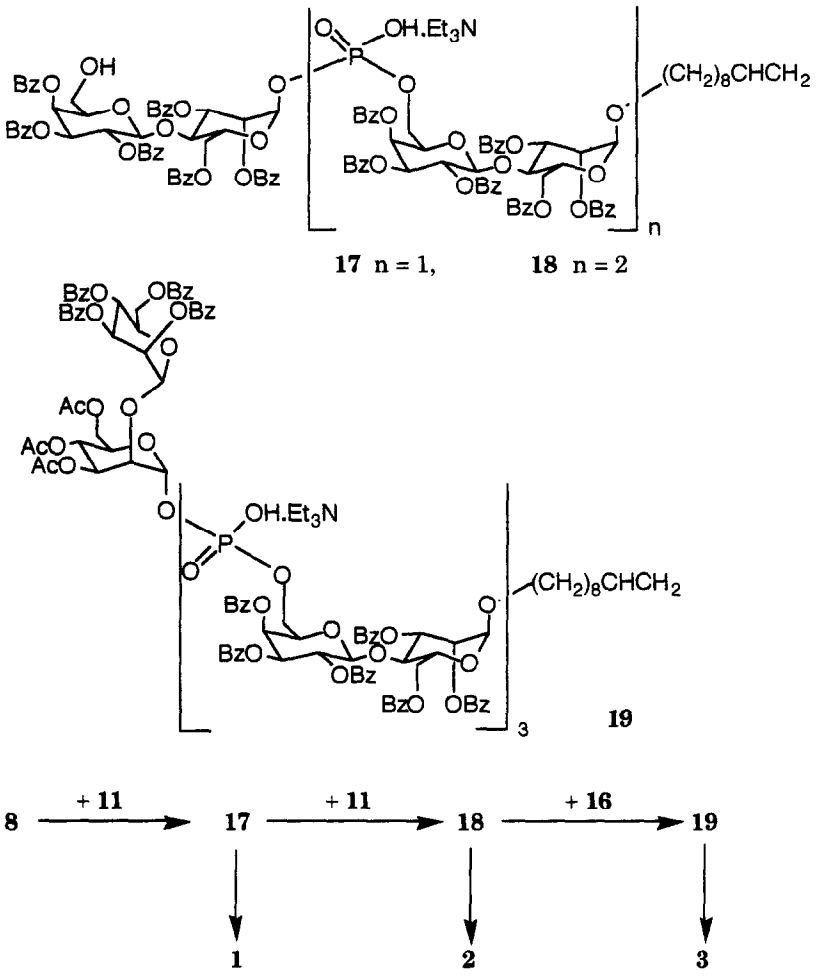
The H-phosphonate block **11**, containing a temporary dimethoxytrityl protecting group at O-6', was prepared from acetobromogalactose and 1,2,3,6-tetra-O-benzoyl-α-D-mannose **5** (ref. 7). The base deficient glycosylation (as above) gave disaccharide **7** (71%), which was converted into **9**



(66%) by consecutive O-deacetylation with $Mg(OMe)_2$ in MeOH[8], dimethoxytritylation and benzoylation. The latter was selectively 1-O-deacylated[2] with Me_2NH in acetonitrile to give the α-OH-derivative **10** (77%), which on phosphitylation[2] with tri-imidazolylphosphine (prepared from PCl_3, imidazole and Et_3N) and mild hydrolysis gave the H-phosphonate derivative **11** (ref. 9) in a yield of 92%.

The base deficient glycosidation (as above) of 1,3,4,6-tetra-O-acetyl-β-D-mannose **13** (purchased from Aldrich) with benzobromomannose **12** furnished the disaccharide **14**, which was converted into the mannobiosyl H-phosphonate **16** (ref. 9; 92% from **13**) by successive 1-O-deacylation, phosphitylation and hydrolysis, as described for **9**.

The elongation cycle for synthesis of the oligomers **1-3** involved coupling of a glycobiosyl H-phosphonate derivative with a hydroxylic acceptor, followed by oxidation of the resulting H-phosphonic diester to the phosphoric diester prior to removal of the temporary dimethoxytrityl



protecting group. Condensation of **11** and **8** in pyridine in the presence of 1-adamantanecarbonyl chloride followed by *in situ* oxidation with I_2 in pyridine-water and mild detritylation (1% TFA/CH_2Cl_2, 0°C) led to the partially benzoylated tetrasaccharide phosphoric diester **17** in an overall yield of 81%. The hexasaccharide diphosphate derivative **18** was prepared in a yield of 75% from **11** and **17** by the sequence of reactions described. On the final step the O-protected octasaccharide triphosphate derivative **19** (ref. 9) was synthesized in a yield of 89% from the mannobiosyl H-phosphonate **16** and **18** using standard procedures for condensation and oxidation. O-Deacylation of **17**, **18**, and **19** with 0.05 M MeONa in MeOH led to the target oligo(glycobiosyl phosphates) **1**, **2**, and **3**, respectively, in the yields greater than 90%.

The structures assigned to the oligomers **1**, **2**, and **3** were supported by NMR and electrospray-MS data. The ^{31}P NMR data[10] are characteristic of glycoside linked phosphoric

diesters[2]. The presence of the (1-6)-phosphodiester linkages was confirmed by the C-1 and C-2 signals of the corresponding mannose units and the C-5 and C-6 signals of the corresponding galactose units in the ^{13}C NMR spectra[11]. The signals were shifted as a result of the α- and β-effects of phosphorylation and coupled with P. The α-configuration of the mannosyl phosphate fragments followed from the the positions of the Man', Man'' and Man''' C-3 and C-5 resonances. The latter were close to the chemical shifts of C-3 and C-5 of α-D-mannopyranosyl phosphate[12] taking into account the influence of the glycosyl substituents at the positions 4 (for Man' and Man'') and 2 (for Man'''). The molecular masses of the oligomers were strictly confirmed by the data of negative charge electrospray-mass-spectra. The main signals in the spectra corresponded to the pseudo-molecular ions for the monophosphate **1** [m/z 883.4, $(M-H)^-$], diphosphate **2** [m/z 643.3, $(M-2H)^{2-}$] and triphosphate **3** [m/z 455.3, $(M-3H)^{3-}$].

To summarize, the first chemical syntheses of fragments (up to octasaccharide) of a natural phosphoglycan, consisting of glycobiosyl phosphate units, have been achieved using the glycosyl H-phosphonate method[2].

Acknowledgements: This work was supported by a Wellcome Trust International Grant (for A.V.N.). We are grateful to Dr. B.N.Green of the VG BIOTECH, Cheshire for electrospray-MS analyses.

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3. The mannoside **4** (mp 85-87°C) was prepared by glycosylation of 9-decen-1-ol with acetobromomannose ($Hg(CN)_2/HgBr_2$, CH_3CN), followed by O-deacetylation and selective O-benzoylation[13] (3 eq. BzCl, pyridine, -20°C).
4. Kovac, P.; Edgar, K.J., *J. Org. Chem.*, **1992**, *57*, 2455.
5. Compounds **4-10**, **14**, and **15** gave both an acceptable elemental analysis and reasonable NMR data. $[\alpha]_D$ values (at 22°C in $CHCl_3$): for **4** +12.5°; for **5** +41°; for **6** -10°; for **7** +26°; for **8** +91.2°; for **9** +62.7°; for **10** +31.5°; for **14** -44°; for **15** -55°.
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7. The compound **5** (mp 183-184°C) was prepared in a yield of 64% by selective O-benzoylation[13] of D-mannose (4 eq. BzCl, pyridine, -40°C).
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9. The structures of **11** and **16-19** were supported by NMR and FAB-MS (or ES-MS) data. $[\alpha]_D$ values (at 22°C in $CHCl_3$): for **11** +24.1°; for **16** -22°; for **17** +70.2°; for **18** +64.5°; for **19** +31.2°.
10. ^{31}P NMR (D_2O) data: for **1-3** δ 1.85, 1.86 and 1.90, respectively. $[\alpha]_D$ values (at 22°C in MeOH): for **1** +40.5°; for **2** +20°; for **3** + 39.5°.
11. ^{13}C NMR (D_2O) data: for **1** δ 65.53 ($J_{c,p}$ ~4 Hz, C-6 of G), 69.89 (C-3 of M'), 71.08 ($J_{c,p}$ 8.3 Hz, C-2 of M'), 73.69 (C-5 of M'), 74.89 ($J_{c,p}$ 7.3 Hz, C-5 of G), 97.12 ($J_{c,p}$ 5.4 Hz, C-1 of M'); for **2** δ 65.64 (2C, $J_{c,p}$ 5.6 Hz, C-6 of G & G'), 69.84 (2C, C-3 of M' & M''), 71.07 (2C, $J_{c,p}$ 7.4 Hz, C-2 of M' & M''), 73.64 (2C, C-5 of M' & M''), 74.93 (2C, $J_{c,p}$ 7.5 Hz, C-5 of G & G'), 97.03 (2C, $J_{c,p}$ 5.6 Hz, C-1 of M' & M''); for **3** δ 65.43 (3C, $J_{c,p}$ 5.2 Hz, C-6 of G-G''), 69.94 (2C, C-3 of M' & M''), 70.67 (C-3 of M'''), 71.05 (2C, $J_{c,p}$ 7.4 Hz, C-2 of M' & M''), 73.51 (2C, C-5 of M' & M''), 74.84 (3C, $J_{c,p}$ 8.1 Hz, C-5 of G-G''), 75.03 (C-5 of M'''), 80.15 ($J_{c,p}$ 7.4 Hz, C-2 of M'''), 95.85 ($J_{c,p}$ 4.6 Hz, C-1 of M'''), 96.99 (2C, $J_{c,p}$ 4,6 Hz, C-1 of M' & M'').
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(*Received in Belgium* 19 *November* 1993; *accepted* 31 *January* 1994)