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Application of MPEG soluble polymer support in the synthesis of oligo-phosphosaccharide fragments from the *Leishmania* lipophosphoglycan

Andrew J. Ross, Irina A. Ivanova, Adrian P. Higson and Andrei V. Nikolaev *

Department of Chemistry, University of Dundee, Dundee DD1 4HN, UK

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Abstract

A polymer (MPEG) supported synthesis of the phosphorylated tetra- and hexa-saccharide fragments of the lipophosphoglycan from *Leishmania* has been developed using mono- and di-saccharide H-phosphonates for construction of the phosphodiester bridges.

Keywords: carbohydrates; polymer support; phosphorous acid and derivatives; phosphoric acids and derivatives.

One of the major molecules forming the glycocalyx of the *Leishmania* parasite is the lipophosphoglycan (LPG), which is produced by the infectious promastigote stage of all species of the parasite. The LPG has been shown to be essential for parasite infectivity and survival[1] thus making the enzymes responsible for its biosynthesis of great interest. It contains a polymeric phosphoglycan region consisting of (1→6)-linked β-D-galactosyl-(1→4)-α-D-mannosyl phosphate repeating units, which has been shown[2] to be assembled in vitro by the sequential action of the *Leishmania* α-D-mannopyranosylphosphate transferase (MPT) and β-D-galactopyranosyl transferase (GT). We have recently described chemical syntheses of oligo-phosphosaccharide substrates[3] and substrate analogues[4] for characterization of the MPT. Here we report the chemical synthesis of the tetraglycosyl diphosphate **1** and hexaglycosyl triphosphate **2**, which are fragments of the LPG containing an α-D-Man*p* phosphate unit at the non-reducing end and are designed to be acceptor substrates for the GT in the *Leishmania*. Both compounds contain a dec-9-enyl aglycone moiety to assist biochemical assays.

α-D-Man*p*-(1-PO$_3$H-[-6)-β-D-Gal*p*-(1→4)-α-D-Man*p*-(1-PO$_3$H-]$_n$-6)-β-D-Gal*p*-O[CH$_2$]$_8$CH:CH$_2$

1 n = 1 **2** n = 2

We have demonstrated previously that oligo-phosphosaccharides composed of glycosyl phosphate repeating units can be prepared using H-phosphonate condensation protocols and stepwise,[3,5] blockwise,[6] or polycondensation[7] chain elongation strategies. However, no polymer-supported synthesis of an oligo(glycosyl phosphate) has been reported so far. Compounds **1** and **2** were prepared in a stepwise

* Corresponding author.

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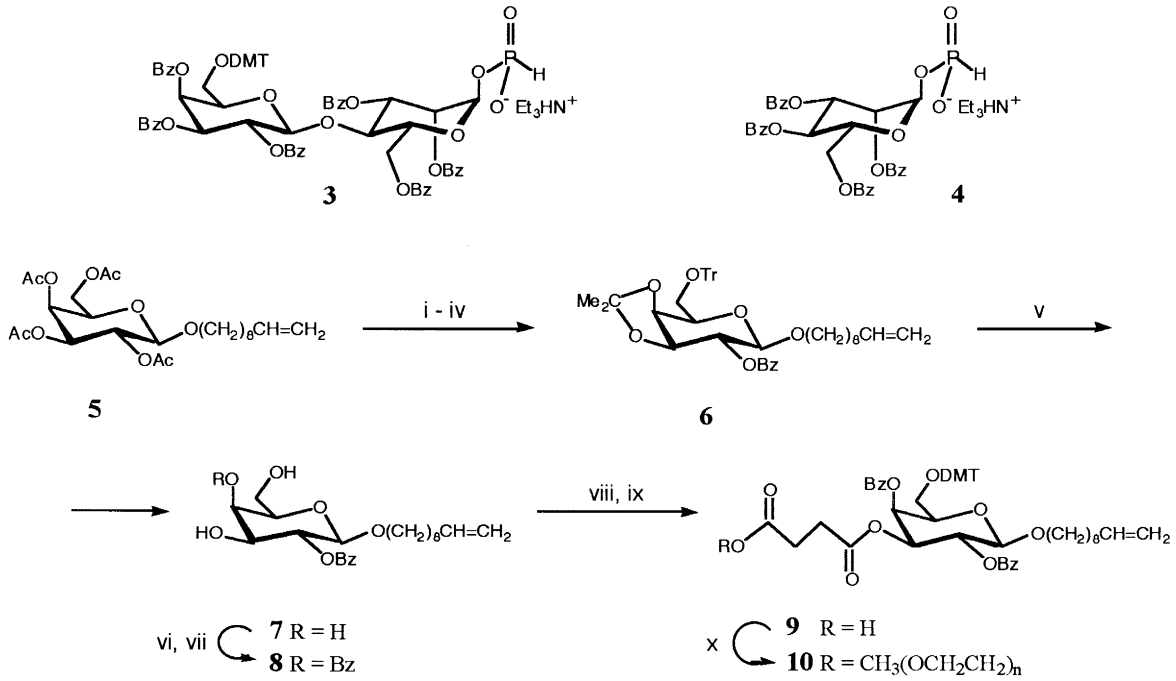
manner using the H-phosphonates **3**[3] and **4**[8] for the chain elongation, the β-D-galactoside derivative **9** (Scheme 1) as a DMT-protected hydroxyl acceptor and monomethyl polyethylene glycol (MPEG, M 5000, loading capacity 0.2 mmol/g) as a polymer support. The advantage of MPEG is that it allows for both solid-phase and solution-phase techniques.[9] MPEG-bound products can be precipitated from ether or crystallized from cold ethanol greatly simplifying their purification. They are, however, soluble in most other organic solvents; this helps to avoid the pseudo-high dilution problems (at the condensation step) associated with solid-phase approaches.



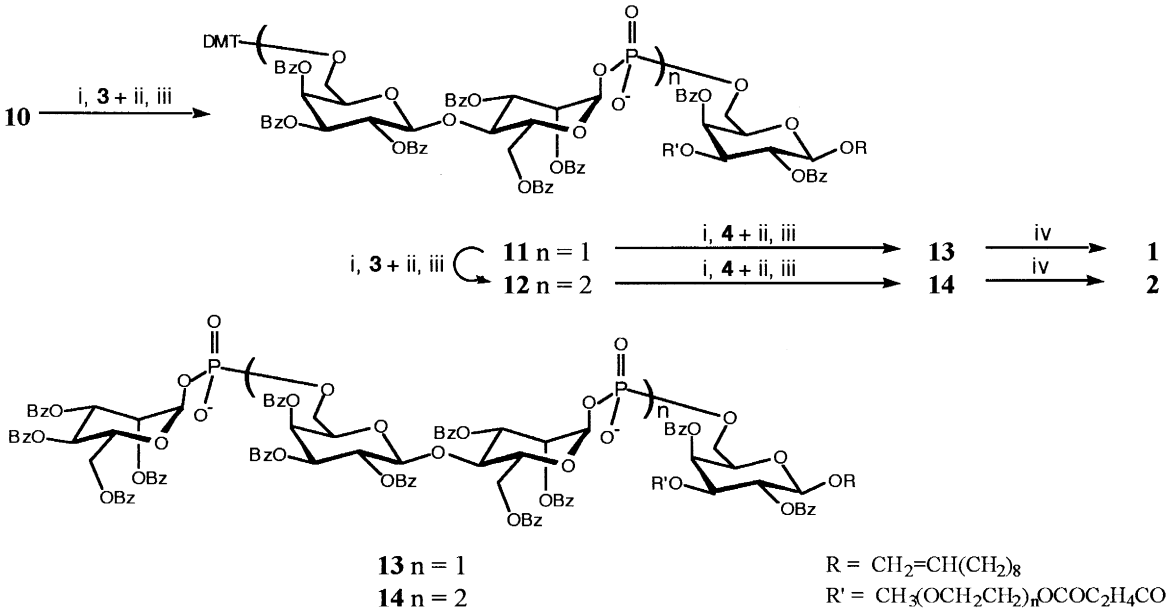
Scheme 1. *Reagents*: (i) NaOMe, MeOH; (ii) TrCl, DMAP, pyridine; (iii) $Me_2C(OMe)_2$, $TsOH \cdot H_2O$, MeCN; (iv) BzCl, pyridine; (v) 80% AcOH, 70°C; (vi) $(EtO)_3CPh$, $TsOH \cdot H_2O$, MeCN; (vii) 80% AcOH, rt; (viii) DMTCl, pyridine; (ix) succinic anhydride, DMAP, pyridine; (x) (a) MPEG, MSNT, 1-methylimidazole, DCM; (b) Ac_2O, pyridine

A succinic diester linker[9] was used for binding the first β-D-galactoside unit **9** to the polymer. It allowed, in combination with benzoic esters (in **3**, **4** and **9**) as permanent *O*-protecting groups, for one-step total deprotection to produce the final products **1** and **2**. The 3-*O*-succinoyl derivative **9** was chosen over its 2-*O*- or 4-*O*-succinoyl isomers to avoid closeness of the linker to either the hydrophobic dec-9-enyl aglycone (that could impede its effective coupling to MPEG) or 6-HO-group (that could hinder effective formation of the (1→6)-phosphodiester linkage). Compound **9** was prepared (Scheme 1) starting from acetobromogalactose and dec-9-en-1-ol, which reacted in the presence of $Hg(CN)_2/HgBr_2$ to give the galactoside **5**[10] (60%). Consecutive deacetylation, 6-*O*-tritylation, treatment with 2,2-dimethoxypropane and conventional benzoylation produced the 2-benzoate **6**[10] (86%). It was converted to the 2,4-dibenzoate **8**[10] (45%) by successive acid hydrolysis (→**7**[10]), reaction with triethyl orthobenzoate to form the corresponding 3,4- and 4,6-orthoesters and their acidic opening, which gives, preferentially,[11] the axial 4-benzoate. Treatment of **8**, first with DMTCl in pyridine and then with succinic anhydride and DMAP resulted in the 6-*O*-DMT-3-*O*-succinoyl-β-D-galactoside **9**[10] in 78% yield.

The derivatization of MPEG began by coupling with **9** in the presence of 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MSNT) and 1-methylimidazole followed by precipitation of the product **10** from ether. The extent of loading **9** on the support was found to be 0.182 mmol/g (91%), as determined

spectrophotometrically[12] at 498 nm after quantitative release of the DMT-cation under acidic conditions. A capping step (Ac_2O/pyridine) was used to block any residual MPEG hydroxyl groups.

A chain elongation cycle for the preparation of the protected oligomers **13** and **14** (Scheme 2) is listed in Table 1. It started by cleavage of the DMT-ether **10** with 1% TFA in DCM followed by neutralization and precipitation of the product from ether. The first elongation step was performed via pivaloyl chloride mediated coupling of the disaccharide H-phosphonate **3** followed by oxidation with iodine to provide the MPEG-bound trisaccharide **11**. It should be noted that a standard work-up procedure is required after the oxidation (see Table 1 and Refs 3–8) to allow the complete precipitation of the product from ethanol. The use of ethanol (not ether) provided the isolation of the pure polymer-bound material (**11**) free of any H-phosphonate contaminants. For the preparation of compound **1**, the above set of procedures and the monosaccharide H-phosphonate **4** were used to end the chain extension (**11**→**13**). Subsequent deprotection (0.1 M methanolic NaOMe) afforded the oligo-phosphosaccharide **1**, which was isolated by anion exchange chromatography[13] in 40% yield based on the galactoside **9** (corresponding to an average 89% yield per step).



Scheme 2. *Reagents*: (i) TFA, DCM; (ii) pivaloyl chloride, pyridine; (iii) I_2, pyridine–water; (iv) NaOMe, MeOH

Table 1
Protocol for the chain elongation cycle

Steps	Reagents and solvents	Time
(1) *Detritylation*	1% TFA/DCM, 0 °C	2 min
Neutralisation	Et_3N-MeOH-DCM (1:2:2)	1 min
Precipitation	ether, 0 °C	
(2) *Coupling*	H-phosphonate (1.5 eq.[a] or 3 eq.[b]), pivaloyl chloride (3 eq.[a] or 6 eq.[b]), pyridine	30 min
(3) *Oxidation*	iodine (3 eq.[a] or 6 eq.[b]), pyridine-water (95:5)	30 min
Work-up	diluting DCM, washing with 0.5 M $Na_2S_2O_3$, then with 0.5 M TEAB	
Crystallisation [c]	ethanol, 0 °C	

[a] The amount used for the synthesis of **1**. [b] The amount used for the synthesis of **2**. [c] In the synthesis of **2**, after the crystallisation, the residual 6-HO-groups were blocked by repeating steps (2) and (3).

The preparation of the hexaglycosyl triphosphate **2** was done in a similar fashion, but was accomplished using an optimized protocol for chain elongation: (1) a double amount of the H-phosphonates

3 and **4** (threefold molar excess instead of 1.5 molar) was used in coupling reactions, and (2) at the end of each cycle, the residual 6-HO-groups of D-galactose were blocked by repeating the condensation and oxidation steps (see Table 1). This enhanced the efficiency of each elongation cycle (determined, as above, spectrophotometrically at 498 nm for the preparation of **11** and **12**; Scheme 2) to 97%. After the final chain extension (→**14**) and total deprotection (0.05 M methanolic NaOMe, 3 h), the oligomer **2** was isolated[13] in 57% yield (corresponding to an average 95% yield per step starting from **9**). The structures of compounds **1** and **2** were confirmed by NMR[14] and electrospray-MS data.[15]

To summarize, the first polymer-supported synthesis of fragments of a natural phosphoglycan composed of glycosyl phosphate units has been performed using the H-phosphonate methodology. A biochemical evaluation of compounds **1** and **2** will be published elsewhere in due course.

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13. Compounds **1** and **2** were isolated on a column of Fractogel TSK DEAE-650 (S), HCO_3^--form, eluted with a linear gradient (0→0.3 M) of NH_4HCO_3 in 40% aq. propan-2-ol. $[\alpha]_D$ values (in degrees, at 24°C in water): for **1** +23.5 (*c* 1); for **2** +19 (*c* 0.3).
14. ^{31}P NMR data (121 MHz, D_2O): for **1** δ_P −2.02; for **2** δ_P −1.97. Selected ^{13}C NMR data (75 MHz, D_2O): for **1** δ_C 61.32 (C-6, Man), 61.92 (C-6, Man′), 65.20 ($J_{C,P}$ 3.4, C-6, Gal), 65.55 ($J_{C,P}$ 3.4, C-6, Gal′), 70.90 ($J_{C,P}$ 8.0, C-2, Man), 71.45 ($J_{C,P}$ 8.0, C-2, Man′), 74.44 ($J_{C,P}$ 8.1, C-5, Gal), 74.80 ($J_{C,P}$ 8.1, C-5, Gal′), 96.93 ($J_{C,P}$ 4.9, C-1, Man), 97.17 ($J_{C,P}$ 4.9, C-1, Man′), 103.82 (C-1, Gal), 104.39 (C-1, Gal′); for **2** δ_C 61.30 (2C, C-6, Man and Man′), 61.90 (C-6, Man′′), 65.20 ($J_{C,P}$ 3.7, C-6, Gal), 65.47 (2C, br, C-6, Gal′ and Gal′′), 70.94 (2C, $J_{C,P}$ 8.1, C-2, Man and Man′), 71.48 ($J_{C,P}$ 7.1, C-2, Man′′), 74.45 ($J_{C,P}$ 8.1, C-5, Gal), 74.77 (2C, $J_{C,P}$ 8.1, C-5, Gal′ and Gal′′), 96.88 (2C, $J_{C,P}$ 4.0, C-1, Man and Man′), 97.18 ($J_{C,P}$ 5.2, C-1, Man′′), 103.79 (C-1, Gal), 104.36 (2C, C-1, Gal′ and Gal′′).
15. The main signals in the ES(−) mass spectra corresponded to the pseudo-molecular ions for the diphosphate **1** (*m/z* 480.90 $[M-2\ H]^{2-}$) and triphosphate **2** (*m/z* 454.95 $[M-3\ H]^{3-}$).