

Synthesis of Novel Glycosyl Phosphate Analogues: Derivatives of an Acceptor Substrate for the *Leishmania* Elongating α -D-Mannopyranosylphosphate Transferase

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Received 1 June 1999; accepted 13 July 1999

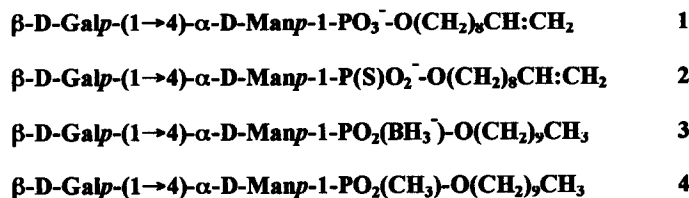
Abstract: The three structural analogues of dec-9-enyl β -D-galactosyl-(1 \rightarrow 4)- α -D-mannosyl phosphate, comprising thiophosphate, boranophosphate and methylphosphonate derivatives, were prepared *via* disaccharide H-phosphonate or trichloroacetimidate (for the methylphosphonate synthesis) intermediates.

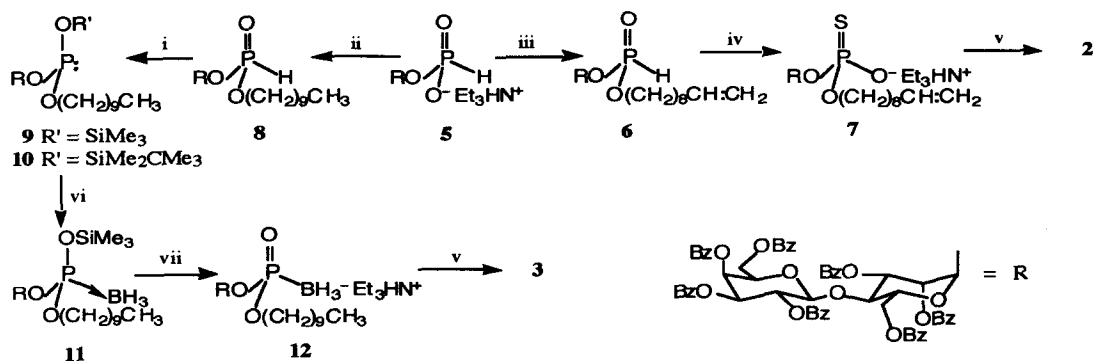
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Keywords: carbohydrates; thiophosphates; boron and compounds; phosphonic acids and derivatives.

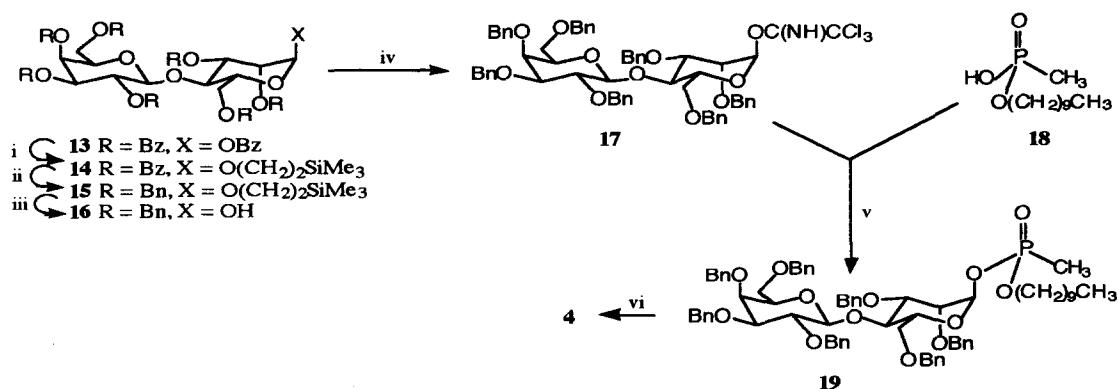
The surface antigenic lipophosphoglycan (LPG) produced by the infectious promastigote stage of all species of the *Leishmania* parasite contains a polymeric section consisting of (1 \rightarrow 6)-linked β -D-galactosyl-(1 \rightarrow 4)- α -D-mannosyl phosphate repeating units. The importance of the LPG for parasite infectivity and survival¹ makes the enzymes responsible for the biosynthesis of this glycoconjugate of great interest. We have recently described chemical syntheses of phospho-oligosaccharide fragments of the LPG of *L. donovani*,² *L. major*³ and *L. mexicana*⁴ and the polymeric phosphoglycan chain of *L. donovani* LPG.⁵ The phosphosaccharides were tested *in vitro* as acceptor substrates for the *Leishmania* α -D-mannopyranosylphosphate transferase (MPT) responsible for the transfer of α -D-mannopyranosylphosphate from GDP-Man to the growing phosphoglycan chain. It has been shown⁶ that the minimum exogenous acceptor substrate structure required for the MPT is the synthetic² phosphosaccharide **1** representing one repeating unit of the phosphoglycan. The presence of a phosphate group in **1** was found to be important for the recognition by the enzyme.

We now report the chemical syntheses of the thiophosphate **2**, boranophosphate **3** and methylphosphonate **4**, structural analogues of compound **1** modified at the phosphate moiety. The data obtained from testing of **2-4** as acceptor substrates of the MPT will be used to gain further information about the enzyme-substrate recognition and to design potential enzyme inhibitors. All the compounds **1-4** contain either a dec-9-enyl, or *n*-decyl moiety that assists biochemical assays.





Scheme 1 Reagents: i, CF₃CON(SiMe₃)₂, THF; ii, *n*-decanol, Me₃CCOCl, pyridine; iii, dec-9-en-1-ol, Me₃CCOCl, pyridine; iv, S₈, toluene-pyridine; v, MeONa, MeOH; vi, BH₃·THF, THF; vii, 1 M Et₃NHCO₃ in water (pH 7).



Scheme 2 Reagents: i, (a) H₂SO₄, Ac₂O; (b) HBr, AcOH-DCM; (c) Me₃Si(CH₂)₂OH, Hg(CN)₂, HgBr₂, MeCN; ii, (a) MeONa, MeOH; (b) BnBr, KOH, DMSO; iii, TFA-DCM (2:1); iv, CCl₃CN, DBU, DCM; v, DCM; vi, H₂, Pd(OH)₂/C, MeOH.

For the preparation of the thiophosphate **2** and boranophosphate **3**, our approach is based on the H-phosphonate method⁷ using the galactosylmannosyl H-phosphonate **5**⁸ (Scheme 1) as a common precursor. Pivaloyl chloride mediated coupling of **5** with dec-9-en-1-ol in pyridine gave the H-phosphonic diester **6**. The following *in situ* oxidation of **6** with sulfur powder⁹ resulted in the protected thiophosphodisaccharide **7**¹⁰ (58%), which was subsequently debenzoylated with 0.025 M MeONa in MeOH to give the thiophosphoric diester **2** (94%) as a mixture of diastereomers [δ_P 53.64 and 53.86 (D₂O)].

To prepare the boranophosphate **3**, the H-phosphonate **5** was first converted to the H-phosphonic diester **8** [δ_P 5.75 and 6.30 (THF)]. Silylation with bis(trimethylsilyl)trifluoroacetamide to form the

Table 1 ^{13}C NMR data [δ_{C} in ppm; $J_{\text{C,P}}$ in Hz (in parentheses); spectra recorded in D_2O] for the phosphoglycosaccharides **1-4**

Residue	Atom	1 ^{a,b}	2 ^b	3 ^b	4 ^{b,c}
Dec-9-enyl or <i>n</i> -decyl	OCH ₂ CH ₂	67.76d (5.1)	68.00d, 68.05d (6.2) (6.2)	64.83d, 65.14d (6.7) (6.7)	66.59d, 66.72d (8.0) (6.6)
	OCH ₂ CH ₂	30.88br	30.71d (6.3)	31.23br	31.23d (7.9)
	-CH= =CH ₂	141.52 115.07	141.47 115.00		
	CH ₃ CH ₂ CH ₃ CH ₂			23.34 14.71	23.67 14.93
Mannose	C-1	96.86br	97.10d, 97.42d (5.6) (5.6)	95.04d, 95.46d (4.2) (4.2)	97.53d, 97.83d (4.2) (3.9)
	C-2	71.20d (7.6)	71.04d, 71.14d (10.4) (10.4)	70.66br, 71.54d (6.7)	70.83br
	C-3	69.73	69.67	69.72	69.69
	C-4	76.97	76.74, 76.86	76.90	76.44, 76.74
	C-5	73.41	73.44, 73.63	73.17	73.75
	C-6	61.18	61.33	61.17	61.01, 61.06
Galactose	C-1	104.12	104.02	104.07	104.18
	C-2	72.04	72.00	72.00	71.95
	C-3	73.62	73.59	73.65	74.42
	C-4	69.82	69.99	69.96	69.69
	C-5	76.46	76.39	76.41	76.44
	C-6	62.20	62.27	62.19	62.20

^a Data are taken from ref. 8.

^b Signals of CCH₂C [δ_{C} 25.96-26.55, 29.10-30.67 and 32.55-34.19] were present.

^c Signals of CH₃P [δ_{C} 10.85, $J_{\text{C,P}}$ 142.34 Hz and 11.45, $J_{\text{C,P}}$ 140.89 Hz for two diastereomers] were present.

trisubstituted phosphite **9** [δ_{P} 127.32 and 129.44 (THF)], followed by “one-pot” boronation¹¹ with $\text{BH}_3 \cdot \text{THF}$ [\rightarrow **11**, δ_{P} 105.13 br (THF)] and hydrolysis of the TMS-ester led to the protected boranophosphodisaccharide **12**¹⁰ [68%, δ_{P} 94.95 br (CDCl_3)]. Debenzoylation (as above) gave the boranophosphate **3** (94%) as a mixture of diastereomers [δ_{P} 92.58 and 94.05 (D_2O)].

For the preparation of the disaccharide methylphosphonate **4**, we attempted, first, to start from the H-phosphonate **8** to form the P-C bond. However, the reaction of the *tert*-butyldimethylsilyl phosphite **10**¹² with MeI or MeSO_3CF_3 resulted in the cleavage of the mannosyl phosphite linkage and gave the corresponding disaccharide hemiacetal derivative as the main product. Therefore, we decided to use decyl methylphosphonic acid **18**¹³ (Scheme 2) and the trichloroacetimidate approach developed by Schmidt.¹⁴ Since the glycosyl methylphosphonate linkage seems to be sensitive to basic treatment, benzyl ethers were used instead of benzoyl esters as permanent protecting groups. The *O*-benzylated biosyl trichloroacetimidate **17** was prepared starting from the *O*-benzoylated disaccharide **13**,⁸ which was converted to the TMS-ethyl bioside **14**¹⁰ (62%) by consecutive acetolysis, 1-bromination and glycosylation of 2-(trimethylsilyl)ethanol in the presence of mercury salts. Debenzoylation of **14** with MeONa in MeOH followed by conventional benzylation led to the

disaccharide derivative **15**, which was treated¹⁵ with TFA-DCM to give the disaccharide hemiacetal **16**¹⁰ (52%). The reaction of **16** with CCl₃CN in the presence of DBU³ gave the trichloroacetimidate **17**¹⁰ in 86% yield. Coupling of **17** and decyl methylphosphonic acid **18** in DCM resulted in a stereoselective formation of the glycosyl methylphosphonate **19**¹⁰ (37%), which gave the methylphosphonodisaccharide **4** [81%, as a mixture of diastereoisomers, δ_p 32.59 and 33.13 (D₂O)] upon hydrogenation over palladium hydroxide on charcoal.

The structures of compounds **2-4** were confirmed by NMR and mass spectrometric data. The ³¹P NMR spectra exhibited signals (see above), which are characteristic of thiophosphoric,⁹ boranophosphoric¹¹ and methylphosphonic¹⁶ diesters, respectively. The structure of the disaccharide fragment was proved by the corresponding signals in the ¹³C NMR spectra of **2-4** (Table 1), which are close to those of the phosphodisaccharide **1**.⁸ The molecular masses of **2-4** were confirmed by electrospray mass spectrometry. The main signals in the ES(-) mass spectra corresponded to the pseudomolecular ions for the thiophosphate **2** (m/z 575.3, [M - Et₃N - H]⁻) and boranophosphate **3** (m/z 559.13, [M - Et₃N - H]⁻). Similarly, the signal in the ES(+) mass spectrum corresponded to the pseudomolecular ion for the methylphosphonate **4** (m/z 583.0, [M + Na]⁺). A biochemical evaluation of compounds **2-4** will be published elsewhere in due course.

Acknowledgements: This work and A. P. H. were supported by a Wellcome Trust Grant 048564/Z/96/Z. One of us (A. J. R.) thanks the BBSRC for the award of a studentship. The research of A. V. N. was supported by an International Research Scholar's award from the Howard Hughes Medical Institute.

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13. Decyl methylphosphonate **18** [δ_p 30.54 (CDCl₃)] was prepared from CH₃POCl₂, imidazole and *n*-decyl alcohol in the presence of Et₃N followed hydrolysis and acidification.
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