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

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

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→6)-linked β-D-galactosyl-(1→4)-α-D-mannosyl phosphate repeating units. The importance of the LPG for parasite infectivity and survival[1] 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 shown[6] that the minimum exogenous acceptor substrate structure required for the MPT is the synthetic[2] 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.

β-D-Gal*p*-(1→4)-α-D-Man*p*-1-PO_3^--O$(CH_2)_8CH{:}CH_2$ **1**

β-D-Gal*p*-(1→4)-α-D-Man*p*-1-$P(S)O_2^-$-O$(CH_2)_8CH{:}CH_2$ **2**

β-D-Gal*p*-(1→4)-α-D-Man*p*-1-$PO_2(BH_3^-)$-O$(CH_2)_9CH_3$ **3**

β-D-Gal*p*-(1→4)-α-D-Man*p*-1-$PO_2(CH_3)$-O$(CH_2)_9CH_3$ **4**

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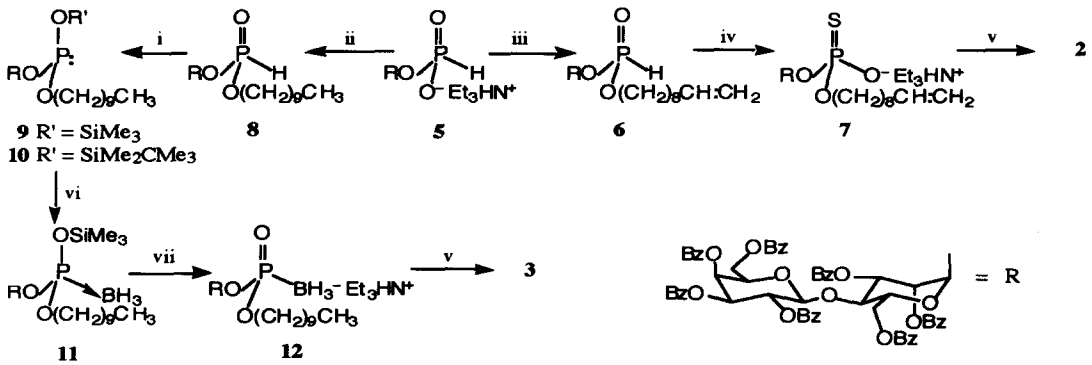
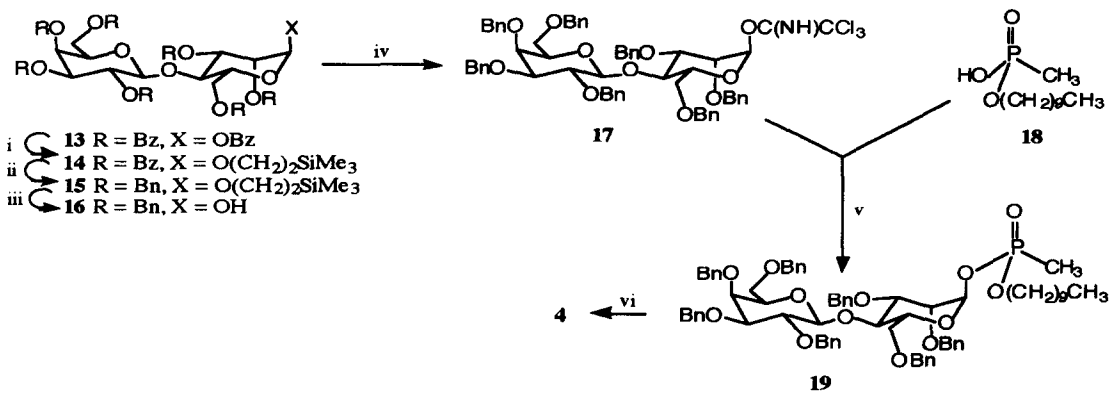


Scheme 1 *Reagents:* i, $CF_3CON(SiMe_3)_2$, THF; ii, *n*-decanol, Me_3CCOCl, pyridine; iii, dec-9-en-1-ol, Me_3CCOCl, pyridine; iv, S_8, toluene-pyridine; v, MeONa, MeOH; vi, BH_3.THF, THF; vii, 1 M Et_3NHHCO_3 in water (pH 7).



Scheme 2 *Reagents:* i, (*a*) H_2SO_4, Ac_2O; (*b*) HBr, AcOH-DCM; (*c*) $Me_3Si(CH_2)_2OH$, $Hg(CN)_2$, $HgBr_2$, MeCN; ii, (*a*) MeONa, MeOH; (*b*) BnBr, KOH, DMSO; iii, TFA-DCM (2:1); iv, CCl_3CN, DBU, DCM; v, DCM; vi, H_2, $Pd(OH)_2/C$, MeOH.

For the preparation of the thiophosphate **2** and boranophosphate **3**, our approach is based on the H-phosphonate method[7] 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 powder[9] 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 (D_2O)].

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; $J_{C,P}$ in Hz (in parentheses); spectra recorded in D_2O] for the phospho-oligosaccharides **1-4**

Residue	Atom	**1**[a,b]	**2**[b]	**3**[b]	**4**[b,c]
Dec-9-enyl or *n*-decyl	OCH_2CH_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_2CH_2	30.88br	30.71d (6.3)	31.23br	31.23d (7.9)
	-CH=	141.52	141.47		
	$=CH_2$	115.07	115.00		
	CH_3CH_2			23.34	23.67
	CH_3CH_2			14.71	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 CCH_2C [δ_C 25.96-26.55, 29.10-30.67 and 32.55-34.19] were present.

[c] Signals of CH_3P [δ_C 10.85, $J_{C,P}$ 142.34 Hz and 11.45, $J_{C,P}$ 140.89 Hz for two diastereomers] were present.

trisubstituted phosphite **9** [δ_P 127.32 and 129.44 (THF)], followed by "one-pot" boronation[11] with BH_3.THF [→**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 consequtive 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 treated[15] with TFA-DCM to give the disaccharide hemiacetal **16**[10] (52%). The reaction of **16** with CCl_3CN in the presence of DBU[3] 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 (D_2O)] upon hydrogenation over palladium hydroxide on charcoal.

The structures of compounds **2-4** were confirmed by NMR and mass spectrometric data. The ^{31}P NMR spectra exhibited signals (see above), which are characteristic of thiophosphoric,[9] boranophosphoric[11] and methylphosphonic[16] diesters, respectively. The structure of the disaccharide fragment was proved by the corresponding signals in the ^{13}C 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, $[M - Et_3N - H]^-$) and boranophosphate **3** (*m/z* 559.13, $[M - Et_3N - H]^-$). 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.

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12. The *tert*-butyldimethylsilyl phosphite **10** [δ_P 126.41 and 128.65 (THF)] was prepared from **8** and TBDMS-chloride in the presence of $(iso\text{-}Pr)_2EtN$.
13. Decyl methylphosphonate **18** [δ_P 30.54 ($CDCl_3$)] was prepared from CH_3POCl_2, imidazole and *n*-decyl alcohol in the presence of Et_3N followed hydrolysis and acidification.
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