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Synthesis of carbohydrate mimetics of an acceptor substrate for the *Leishmania* elongating α-D-mannopyranosylphosphate transferase

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Abstract

Two mimetics of dec-9-enyl β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl phosphate containing the phosphodiester (**3**) or phosphonate (**4**) groups have been synthesized. © 2000 Elsevier Science Ltd. All rights reserved.

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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. The polymeric phosphoglycan region of the LPG was shown[2] to be assembled in vitro by the sequential action of the *Leishmania* α-D-Man*p*-phosphate transferase (MPT) and β-D-Gal*p* transferase (GT) using GDP-Man and UDP-Gal, respectively, as substrate donors. Since there are no other mannosylphosphate transferases in mammals, the *Leishmania* MPT seems to be a good drug target. A fine substrate specificity of the MPT has been recently studied using synthetic oligo-phosphosaccharide substrates[3] and substrate analogues.[4] The results[5,6] showed that: (1) the phosphodisaccharide **1** representing one repeating unit of the phosphoglycan is an efficient acceptor substrate for the MPT; (2) the negative charge of the phosphodiester group is essential for the enzyme recognition; (3) both the C-6 and C-6′ primary hydroxyls of **1** are essential for substrate recognition as well as the C-6′ hydroxyl (the acceptor site) is essential for catalysis; and (4) C-2, C-3, C-2′, C-3′ and C-4′ hydroxyls of **1** make little, or no contribution to the substrate recognition.

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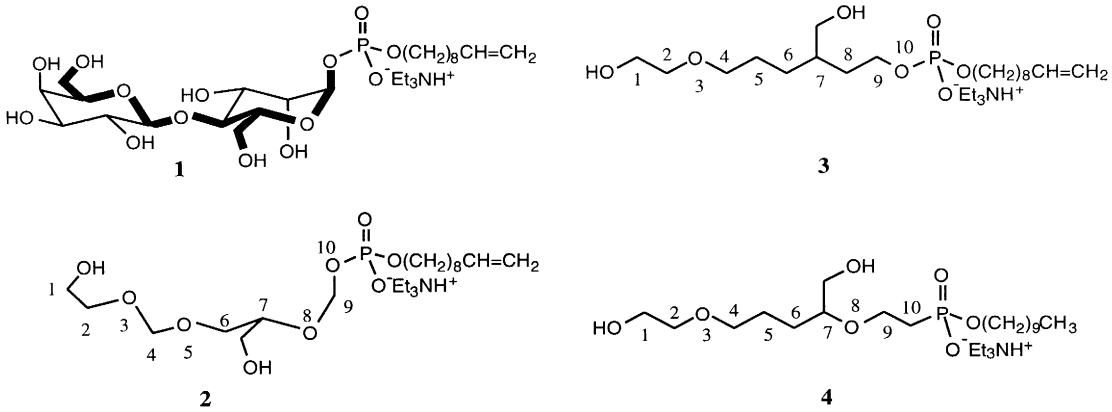
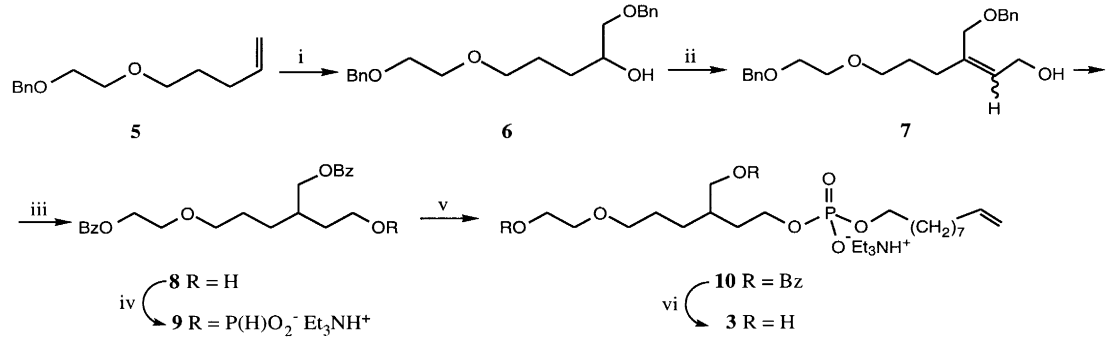


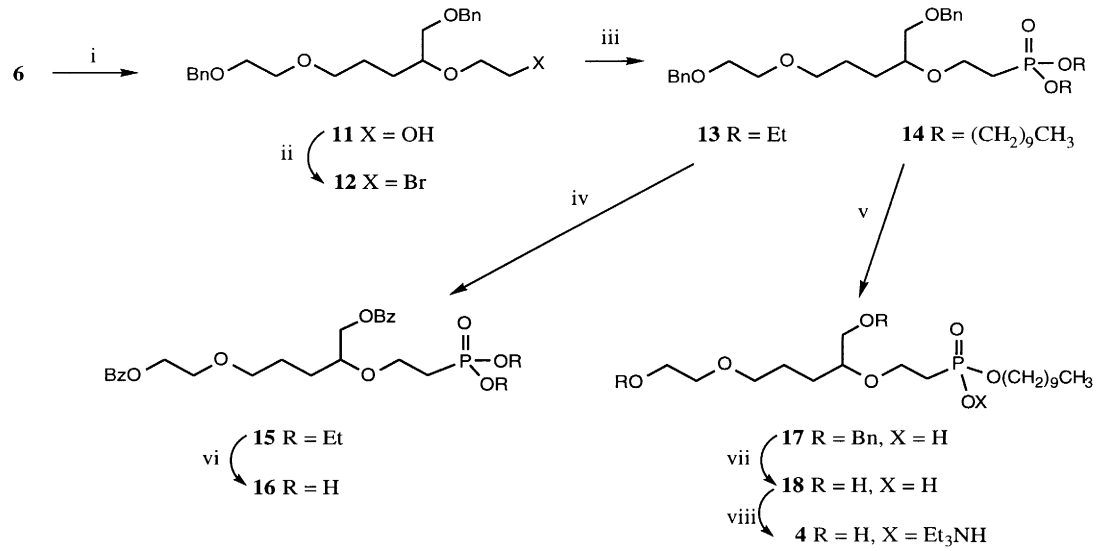
Here, we report the synthesis of two structural mimetics **3** and **4** of the MPT substrate **1** containing just the elements of the structure, which were shown to be necessary for the enzyme recognition (i.e. both primary hydroxyls and the phosphate anion). To secure chemical stability of the mimetics, the intersaccharidic oxygen (O-5 in the hypothetical mimetic analog **2**) was replaced with the CH_2-group and the O–C–O–P segment (O-8–C-9–O-10–P in the structure **2**) was replaced with either C-8–C-9–O-10–P (in **3**), or O-8–C-9–C-10–P (in **4**) fragments. The data obtained from testing **3** and **4** as acceptor substrates for the MPT will be used to gain more comprehensive information about the substrate specificity of the enzyme and to design potential inhibitors. Both compounds contain either a dec-9-enyl, or a *n*-decyl moiety that assists biochemical assays.

The phosphoric diester **3** was obtained from the alcohol **8** (Scheme 1) and dec-9-en-1-ol using the H-phosphonate method.[7] The phosphonic ester **4** was synthesized using the Arbusov reaction[8] from the alkyl bromide **12** (Scheme 2) and tri-*n*-decyl phosphite. A common intermediate for both targeted compounds, the alcohol **6**, was prepared starting from commercially available 2-benzyloxyethanol and 5-bromopent-1-ene. Their reaction in DMF in the presence of sodium hydride gave the ether **5**. Successive osmium tetroxide catalyzed hydroxylation[9] of the double bond yielded the corresponding diol, which was then selectively protected with benzyl bromide via a stannylene intermediate[10] to afford the secondary alcohol **6** in 46% overall yield (based on 2-benzyloxyethanol).



Scheme 1. Reagents: (i) (a) OsO_4 cat., NMO, acetone–water; (b) Bu_2SnO, $PhCH_3$, Δ; (c) BnBr, *n*-Bu_4NI; (ii) (a) *n*-Pr_4NRuO_4 cat., NMO, MS 4 Å, CH_2Cl_2; (b) NaH, $(MeO)_2POCH_2COOMe$, THF; (c) $LiAlH_4$, THF; (iii) (a) TBDMSCl, imidazole, DMF; (b) H_2, Ra–Ni; (c) BzCl, pyridine; (d) *n*-Bu_4NF, THF; (iv) (a) tri-imidazolylphosphine, MeCN; (b) Et_3NHHCO_3, water (pH 7); (v) (a) dec-9-en-1-ol, Me_3CCOCl, pyridine; (b) I_2, pyridine–water; (vi) NaOMe, MeOH

For the preparation of **3**, the derivative **6** was first oxidized[11] to the corresponding ketone with *N*-methylmorpholine *N*-oxide (NMO) in the presence of *n*-Pr_4NRuO_4 followed by the Horner–Emmons olefination[12] with trimethyl phosphonoacetate and reduction with $LiAlH_4$ to produce the allylic alcohol



Scheme 2. Reagents: (i) (a) NaH, allyl bromide, DMF; (b) OsO_4 cat., NMO, acetone–water, then Na_2SO_3, then $NaIO_4$; (c) $NaBH_4$, EtOH; (ii) (a) $MeSO_2Cl$, Et_3N, CH_2Cl_2; (b) *n*-Bu_4NBr, $PhCH_3$, Δ; (iii) $P(OEt)_3$, Δ, for **13**; $P(OC_{10}H_{21}$-*n*$)_3$, Δ, for **14**; (iv) (a) H_2, $Pd(OH)_2$/C, MeOH; (b) BzCl, pyridine; (v) NaOH, dioxane–water, Δ; (vi) TMSI, CH_2Cl_2; (vii) H_2, $Pd(OH)_2$/C, MeOH; (viii) Et_3N, MeOH

7 (87%) as a mixture of two isomers. It was then converted to the alcohol **8** (89%) (representing the disaccharide fragment in the mimetic structure **3**) by consecutive *O*-protection with TBDMS–chloride, hydrogenation of the double bond and both benzyl ethers over a nickel catalyst, conventional *O*-benzoylation and desilylation with TBAF. Phosphitylation[3,4,7] of **8** with tri-imidazolylphosphine (prepared in situ from PCl_3, imidazole and Et_3N) followed by mild hydrolysis gave the H-phosphonate **9**, which produced the protected phosphoric diester **10** (73%, δ_P −0.46) upon condensation[3,4,7] with dec-9-en-1-ol in pyridine in the presence of trimethylacetyl chloride and oxidation of the resulting phosphorous diester with iodine in aq. pyridine. Debenzoylation of **10** with 0.1 M methanolic sodium methoxide provided the phosphodiester **3**[13] quantitatively.

For the preparation of **4** (Scheme 2), the alcohol **6** was first allylated with allyl bromide in the presence of sodium hydride to give the corresponding allyl ether, which was then transformed to the alcohol **11** (93% based on **6**) by consecutive hydroxylation with OsO_4, periodate cleavage of the resulting diol and reduction of the intermediate aldehyde with $NaBH_4$. Conventional mesylation of the alcohol **11** followed by bromination of the mesylate with *n*-Bu_4NBr led to the key 2-alkoxy-ethyl bromide **12** (95%). The Arbusov reaction of the bromide **12** with tri-*n*-decyl phosphite[14] (neat, 170°C) afforded the phosphonic diester **14** (73%, δ_P 28.7), which was selectively hydrolyzed[15] by refluxing with 2% NaOH in dioxane–water to produce (after neutralization with 0.5 M HCl) the *n*-decyl phosphonate **17** (H^+-form, δ_P 30.8) in 56% yield (93% based on recovered **14**). Final debenzylation of **17** by hydrogenation over $Pd(OH)_2$/C (→**18**)[16] followed by neutralization with triethylamine provided the phosphonic acid monoester **4**[17] quantitatively.

Alternatively, we attempted to prepare the ester **4** via the phosphonic acid **16**. The Arbusov reaction of the bromide **12** with triethyl phosphite[18] (neat, 150°C) led to the diethyl phosphonate **13** (δ_P 28.8) in 92% yield. The dibenzyl ether **13** was then *O*-reprotected by catalytic hydrogenation followed by conventional benzoylation to give the dibenzoate **15** (88%, δ_P 28.5). Subsequent *P*-deprotection of **15** with TMSI[18] in CH_2Cl_2 at 0°C afforded the phosphonic acid **16**[19] (100%). Regrettably, all attempts to convert it to the corresponding mono-*n*-decyl phosphonate by esterification[20] with *n*-decanol in the presence of DCC, or other condensing agents (TPS-NT, MS-NT, HATU, etc.) failed.

The carbohydrate mimetics **3** and **4** are being tested as substrates for the *Leishmania* α-D-Man*p*-phosphate transferase and the results will be published elsewhere in due course.

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13. Compound **3**: δ_H (D_2O) 0.90–1.80 (21H, m, 10×CH_2 and CH), 0.96 (9H, t, *J*=7.3, 3×C*H*$_3$CH$_2$N), 2.88 (6H, q, 3×CH$_3$C*H*$_2$N), 3.17–3.30 (6H, m, 3×CH_2O), 3.38 (2H, m, CH_2O), 3.53 and 3.59 (4H, 2×q, *J*=6.3, 2×CH$_2$C*H*$_2$OP), 4.63 (1H, br d, J_{cis}=10.2, *cis*-H from C*H*$_2$=CH), 4.70 (1H, br d, J_{trans}=17.8, *trans*-H from C*H*$_2$=CH) and 5.52 (1H, ddt, $J_{H,CH2}$=6.7, CH$_2$=C*H*CH$_2$); δ_C (D_2O) 8.6 (NCH$_2$*C*H$_3$), 25.8, 26.4, 27.1, 29.0, 29.2, 29.3 and 29.5 (7×CH_2), 30.5 and 31.9 (2×d, $J_{C,P}$=6.5, 2×*C*H$_2$CH$_2$OP), 33.9 (CH$_2$=CH*C*H$_2$), 36.8 (CH), 47.0 (NCH_2), 60.8 and 64.2 (2×CH_2OH), 64.3 and 66.4 (2×d, $J_{C,P}$=5.1, 2×CH_2OP), 71.6 and 71.8 (CH_2OCH_2), 114.5 (*C*H$_2$=CH) and 139.7 (CH$_2$=*C*H); δ_P (D_2O) 0.70; ES-MS (−) data: *m/z* 409.21 (100%, [M–Et$_3$N–H]$^-$), ($C_{25}H_{54}NO_7P$ requires *M*, 511.36).
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16. Compound **18**: δ_C ($CDCl_3$) 14.5 (CH_3), 23.1, 25.8, 29.6, 29.7, 29.9 (2C) and 32.3 [(*C*H$_2$)$_7$CH$_3$], 25.9 and 28.1 [OCH$_2$(*C*H$_2$)$_2$CHO], 27.8 (d, $J_{C,P}$=142.1, CH_2P), 30.9 (d, $J_{C,P}$=5.1, *C*H$_2$CH$_2$OP), 62.1 and 64.5 (2×CH_2OH), 64.0 and 65.8 (2×br, *C*H$_2$CH$_2$PO*C*H$_2$), 71.5 and 72.4 (CH_2OCH_2) and 81.3 (CHO); δ_P ($CDCl_3$) 31.1.
17. Compound **4**: δ_H (CD_3OD) 0.92 (3H, t, *J*=6.9, CH_3 from *n*-decyl), 1.28–1.80 [29H, m, (C*H*$_2$)$_8$CH$_3$, 3×C*H*$_3$CH$_2$N and OCH$_2$(C*H*$_2$)$_2$CHO], 1.94 (2H, dt, $J_{H,H}$=7.8, $J_{H,P}$=17.3, OCH$_2$C*H*$_2$P), 3.21 (6H, q, *J*=7.1, 3×CH$_3$C*H*$_2$N), 3.30–3.90 (11H, m, 5×CH_2O and CHO) and 3.85 (2H, q, *J*=6.2, CH$_2$C*H*$_2$OP); δ_P (CD_3OD) 21.9; ES-MS (−) data: *m/z* 411.24 (100%, [M–Et$_3$N–H]$^-$), ($C_{25}H_{56}NO_7P$ requires *M*, 513.38).
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19. Compound **16**: δ_C ($CDCl_3$) 25.7 and 28.6 [OCH$_2$(*C*H$_2$)$_2$CHO], 28.2 (d, $J_{C,P}$=137.0, CH_2P), 64.3 and 65.7 (2×*C*H$_2$OBz), 65.5 (br, *C*H$_2$CH$_2$P), 68.8 and 71.3 (CH_2OCH_2), 78.1 (CHO), 128.4–133.3 (Ph), 166.7 and 167.0 (2×C=O); δ_P ($CDCl_3$) 24.25.
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