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Synthesis of β-D-Gal*p*-(1→4)-α-D-Man*p* methanephosphonate, a substrate analogue for the elongating α-D-mannosyl phosphate transferase in the *Leishmania*

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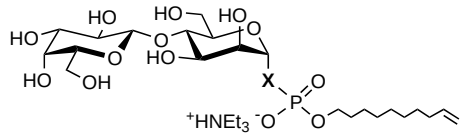
Abstract—An isosteric *C*-glycoside phosphono analogue of dec-9-enyl β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl phosphate was synthesised and showed high biological activity in the *Leishmania* MPT assay. A one-step Horner–Emmons/Michael reaction was developed for the stereoselective preparation of α-D-mannosyl methanephosphonate derivatives.

Recent reports from this laboratory disclosed the synthesis and biological evaluation of β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl phosphate **1** and a number of its structural analogues.[1,2] The parent compound was shown to be an efficient exogenous acceptor substrate for elongating α-mannosyl phosphate transferase (MPT) in the three species of *Leishmania*, while biochemical assays performed with structural analogues of **1** helped to define the true acceptor substrate specificity of the enzyme.[3] As the next step of the project, which is targeting the preparation of selective bisubstrate inhibitors[4] of the MPT, a demand for hydrolytically stable analogues[5] of compound **1** has been recognised. Here we report the chemical synthesis of the isosteric *C*-glycoside phosphono analogue **2**, which then was tested as an acceptor substrate for the MPT.



1 X = O
2 X = CH_2

A retrosynthetic analysis exposed the preparation of a selectively protected α-D-mannosyl methanephosphonate derivative as the key problem. The rest of the synthetic plan comprised 4-*O*-glycosylation of the above mannosyl methanephosphonate with a suitable galactosyl donor and formation of phosphonic acid dec-9-enyl ester.

Although syntheses of phosphono analogues of natural glycosyl phosphates are well documented,[6] no reliable approach to α-D-mannosyl methanephosphonate derivatives has been reported.[7] The closest analogy available was the preparation of diethyl (α-L-rhamnosyl methanephosphonate) via one-step Horner–Emmons/Michael reaction of tetraethyl methylenediphosphonate sodium with 2,3,4-tri-*O*-benzyl-α,β-L-rhamnose.[8] In our hands, however, the reaction of 2,3,4,6-tetra-*O*-benzyl-D-mannose **3** with tetramethyl methylenediphosphonate **4** and NaH yielded the conjugated vinylphosphonate **5** exclusively (Scheme 1).[9] Other combinations of solvents and bases provided the same product, except LiHMDS in THF at 55°C: this time the desired α-D-mannosyl methanephosphonate derivative **6**[10] was isolated in 33% yield accompanied by the isomerised starting material **7** (50%).

To exclude the unwanted elimination of the 3-benzyloxy group, 2,3-*O*-isopropylidene-D-mannose derivatives were tested in the above Horner–Emmons/Michael reaction. To this end, the allyl mannoside **9** prepared by $BF_3 \cdot Et_2O$ mediated mannosylation of allyl alcohol with D-mannose penta-acetate **8** was effectively processed

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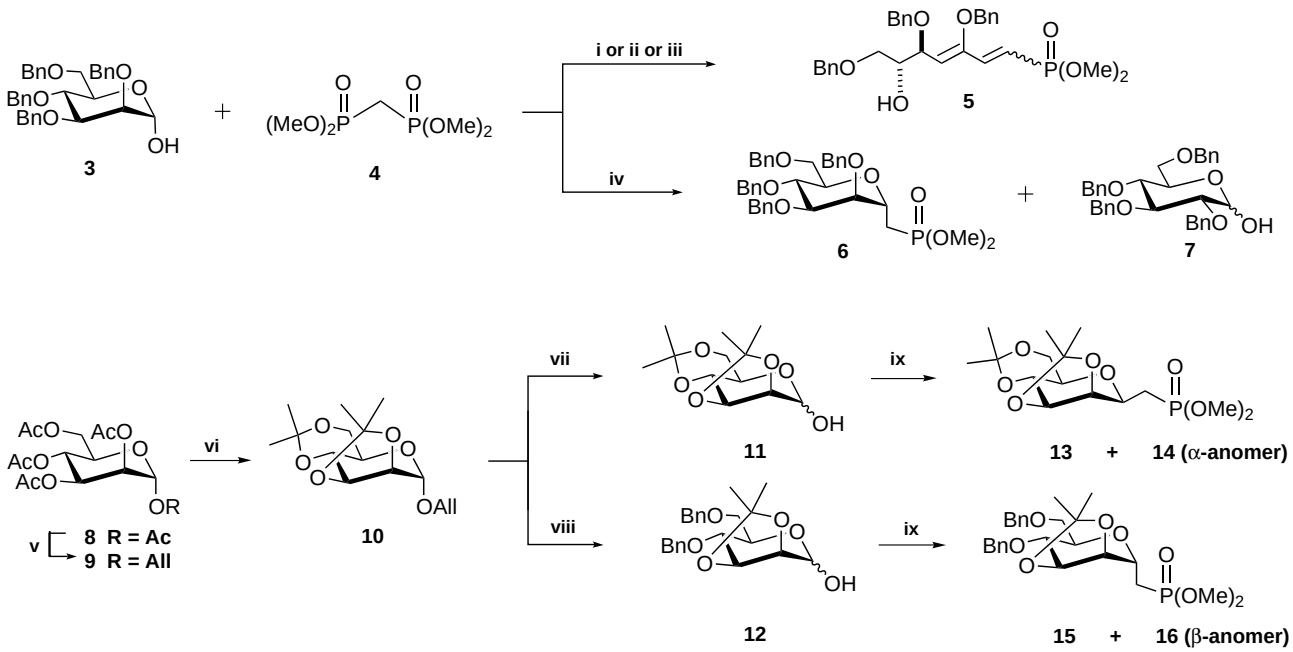
into differentially protected hemiacetals **11** and **12** via the common diisopropylidenated precursor **10** (Scheme 1).

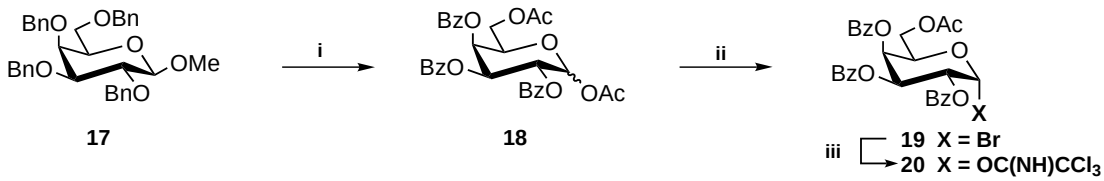
Treatment of the bis-isopropylidene hemiacetal **11** with **4** and LiHMDS in THF at 55°C produced a mixture of α- and β-D-mannosyl methanephosphonates **14** and **13**, respectively, in 80% yield with the β-anomer **13**[10] predominating (α:β = 1:2.5). Similar reaction of the monoisopropylidene derivative **12** gave a separable mixture of the methanephosphonates **15** and **16** with a prevalence of the α-anomer **15**[10] (α:β = 1.7:1). No elimination product was detected in either case. Base screening led to an improvement of the **15**/**16** ratio up to the very acceptable 10:1 using *t*-BuOK in THF. The desired α-D-mannosyl methanephosphonate **15** was isolated in 85% yield. For the **14**/**13** ratio, the figure did not exceed 2.8:1 with the same reagent. These experiments demonstrated that either of the D-mannosyl methanephosphonate anomers could be obtained stereoselectively from D-mannose hemiacetal derivatives. The stereo outcome depends on the protective group pattern and proper choice of the reaction conditions.

The glycosylation reaction was the next step we addressed. To ensure selective handling of the 6-OH group in the galactosyl residue required for further functionalisation at this position, judiciously protected galactosyl donors were needed. These were prepared by the acetolysis[11] and further protective group modification of methyl tetra-*O*-benzyl-β-D-galactopyranoside **17** which provided an anomeric mixture of 1,6-diacetates **18**, which were further transformed into galactosyl bromide **19** and trichloroacetimidate **20** (Scheme 2).

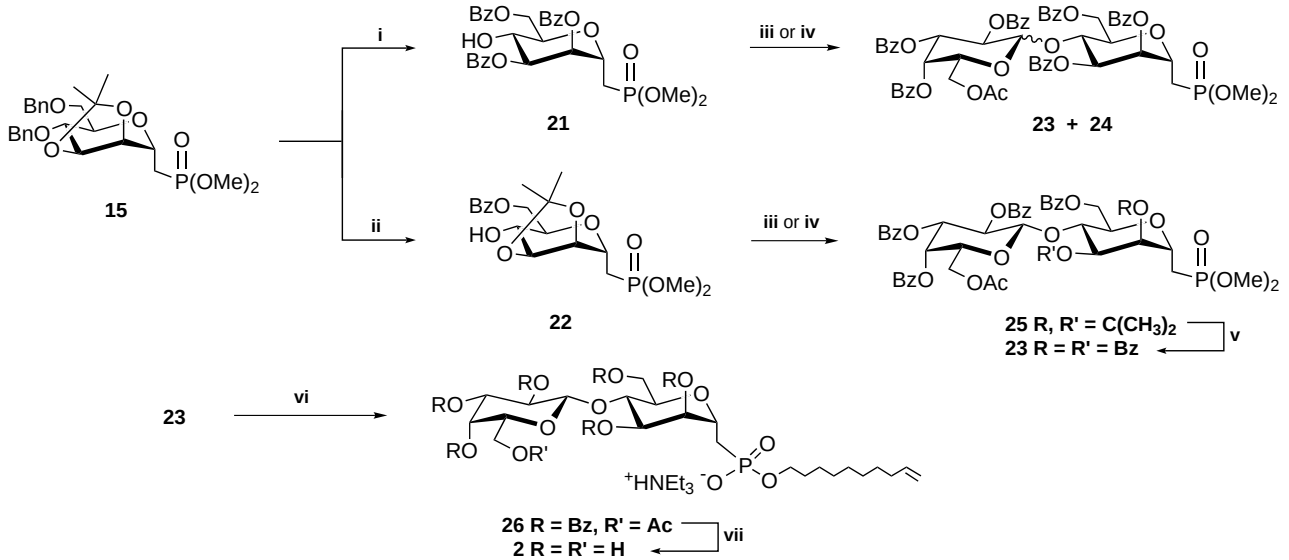
Two differently protected glycosyl acceptors **21** and **22** were synthesised from the methanephosphonate **15** in a straightforward manner (Scheme 3). Selective benzoylation of the primary hydroxyl group was successfully accomplished with benzoyl cyanide in both cases. The presence of the 2,3-*O*-isopropylidene group in the glycosyl acceptor **22** was found to influence dramatically the stereoselectivity of the glycosylation reaction. Indeed, only the required β-linked disaccharide **25** (75–78% yield, $J_{1,2} = 8.0$ Hz) was formed in the reaction of compound **22** with either galactosyl bromide **19** or trichloroacetimidate **20**. In contrast, an inseparable



Scheme 1. *Reagents and conditions*: (i) NaH, diglyme or THF, 12 h; (ii) KHMDS, THF, 20 h; (iii) KOBu-*t*, THF, 2 h; (iv) LiHMDS, THF, 55°C, 4 h; (v) AllOH, $BF_3{\cdot}Et_2O$, 18 h, 80%; (vi) (a) MeOH, MeONa cat., 94%; (b) Me_2CO, $Me_2C(OMe)_2$, $TsOH{\cdot}H_2O$ cat., 90%; (vii) KOBu-*t*, DMSO, 60°C, 1 h, then $Hg(OAc)_2$, THF–H_2O, 20 min, 97%; (viii) (a) AcOH–H_2O 8:2, 12 h, 0°C, 78%; (b) NaH, DMF, BnBr, 2 h, 92%; (c) KOBu-*t*, DMSO, 60°C, 1 h, then $Hg(OAc)_2$, THF–H_2O, 20 min, 95%; (ix) **4**, base, THF; see text for details.



Scheme 2. *Reagents and conditions*: (i) (a) Ac_2O–AcOH, H_2SO_4, 18 h, 80%; (b) $H_2/Pd(OH)_2$-C, MeOH; (c) BzCl, pyridine, 80%; (ii) (a) HBr/AcOH, Ac_2O, DCM, 85%; (iii) (a) Ag_2CO_3, acetone–water; (b) Cl_3CCN, DBU, DCM, 85%.



Scheme 3. *Reagents and conditions*: (i) (a) AcOH–H_2O 8:2, 60°C, 3 h; (b) BzCl, pyridine, 78% for a, b; (c) H_2/$Pd(OH)_2$-C, MeOH, 95%; (d) BzCN, Et_3N, MeCN–DCM, −30°C, 30 min, 78%; (ii) (a) H_2/$Pd(OH)_2$-C, MeOH; (b) BzCN, Et_3N, MeCN–DCM, −30°C, 30 min, 73%; (iii) **19**, AgOTf, *sym*-collidine, DCM, −30°C, 40 min, 80% for **19**+**21**, 75% for **19**+**22**; (iv) **20**, TMSOTf, 4 Å MS, DCM, −30°C, 2 h, 86% for **20**+**21**, 78% for **20**+**22**; (v) (a) $C_5H_5N{\cdot}HClO_4$, CH_3NO_2–MeOH, 65°C, 8 h; (b) BzCl, DMAP, pyridine, 80%; (vi) (a) TMSBr, *sym*-collidine, DCM–CH_3CN; (b) dec-9-en-1-ol, DCC, pyridine, 36 h, 58%; (vii) MeOH, MeONa cat., quantitative.

mixture of α- and β-anomers **24** and **23**, respectively (α:β = 1:2 in each case) was isolated from the reaction of 2,3,4-tribenzoate **21** with the same glycosyl donors. Finally, the isopropylidene group in compound **25** was replaced with benzoyl protection to yield the disaccharide methanephosphonate **23**.

Demethylation of compound **23** with TMSBr in the presence of *sym*-collidine as an acid scavenger[12] afforded the corresponding phosphonic acid derivative, which was then coupled with dec-9-enol in the presence of DCC[13] to give the protected phosphonic mono-ester **26** (δ_P 16.7) in a reliable 55–60% yield (Scheme 3). Deacylation of **26** with catalytic sodium methoxide in MeOH provided the targeted disaccharide methanephosphonate dec-9-enyl ester **2**[14] quantitatively. This compound was tested in a biochemical assay[3] and was shown to be an effective substrate acceptor for the MPT in *Leishmania donovani*: compound **2** revealed 84(±3)% acceptor substrate activity compared with the disaccharide phosphate **1**.

Acknowledgements

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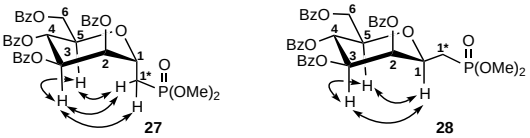
9. All new compounds showed satisfactory spectral and analytical data.
10. The anomeric configurations of compounds **6**, **14**, **15** (α-D-anomers) and **13**, **16** (β-D-anomers) were deduced

from the NOESY spectra of their perbenzoylated derivatives **27** and **28**, respectively (characteristic NOE contacts are shown by the curved arrows at the diagram below).



A mixture of **13** and **14** was converted to compounds **27** and **28** in two steps: (a) AcOH–H_2O, 60°C; (b) BzCl, pyridine, DMAP; followed by separation on silica gel. Compound **27** was also prepared from **15** in three steps: (a) H_2, $Pd(OH)_2$/C, MeOH; (b) Dowex-50 (H^+), MeOH; (c) BzCl, pyridine, DMAP, and from **6** in two steps: (a) H_2, $Pd(OH)_2$/C, MeOH; (b) BzCl, pyridine, DMAP. Compound **28** was obtained from **16** as described for the preparation of **27** from **15**.

27: ^{1}H NMR (300 MHz, C_6D_6): δ 2.02 (ddd, 1H, $J_{1*a,1*b}=15.3$, $J_{1*a,1}=5.5$, $J_{1*a,P}=15.3$, H-1*a), 2.30 (ddd, 1H, $J_{1*b,1}=9.7$, $J_{1*b,P}=18.0$, H-1*b), 3.57 (d, 6H, $J_{H,P}=11.0$, 2×OCH_3), 4.48 (ddd, 1H, H-5), 4.70 (dd, 1H, $J_{5,6a}=3.7$, $J_{6a,6b}=12.0$, H-6a), 4.85 (ddd, 1H, $J_{1,P}=9.0$, H-1), 5.05 (dd, 1H, $J_{5,6b}=2.5$, H-6b), 6.12 (br, 1H, H-2), 6.15 (dd, 1H, $J_{2,3}=3.0$, H-3), 6.62 (t, 1H, $J_{3,4}=J_{4,5}=9.0$, H-4), 7.30–8.30 (m, 20H, aromatic); ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.5 (d, $J_{C,P}=143.8$, C-1*), 52.9 (d, 2C, $J_{C,P}=6.5$, 2×OCH_3), 62.6 (C-6), 67.3 (C-4), 69.5 (C-3), 70.6 (d, $J_{C,P}=3.9$, C-1), 71.1 (C-5), 71.2 (d, $J_{C,P}=15.6$, C-2); ^{31}P NMR (121 MHz, $CDCl_3$): δ 28.0; $[\alpha]_D$ −73.8 (*c* 1.22, $CHCl_3$).

28: ^{1}H NMR (300 MHz, C_6D_6): δ 2.05 (dt, 1H, 15, $J_{1*a,1}=4.5$, $J_{1*a,1*b}=J_{1*a,P}=15.0$, H-1*a), 2.30 (ddd, 1H, $J_{1*b,1}=9.0$, $J_{1*b,P}=17.0$, H-1*b), 3.47 (d, 3H, $J_{H,P}=11.0$, OCH_3), 3.60 (d, 3H, $J_{H,P}=11.0$, OCH_3), 3.80 (ddd, 1H, $J_{4,5}=10.0$, H-5), 4.40 (dd, 1H, $J_{5,6a}=4.0$, $J_{6a,6b}=12.0$, H-6a), 4.50 (ddd, 1H, $J_{1,P}=9.0$, H-1), 4.96 (dd, 1H, $J_{5,6b}=2.3$, H-6b), 5.92 (dd, 1H, $J_{2,3}=3.0$, H-3), 6.15 (d, 1H, H-2), 6.55 (t, 1H, $J_{3,4}=J_{4,5}=10.0$, H-4), 7.30–8.30 (m, 20H, aromatic); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.5 (d, $J_{C,P}=143.7$, C-1*), 52.2 (d, $J_{C,P}=6.0$, OCH_3), 52.8 (d, $J_{C,P}=6.0$, OCH_3), 62.9 (C-6), 66.4 (C-4), 70.7 (d, $J_{C,P}=11.1$, C-2), 72.7 (C-1), 72.9 (C-3), 76.3 (C-5); ^{31}P NMR (121 MHz, $CDCl_3$): δ 29.0; $[\alpha]_D$ −105.6 (*c* 1.02, $CHCl_3$).

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14. **2**: ^{13}C NMR (75 MHz, D_2O): δ 8.5 (Et_3N), 25.3 (C*C*H_2C), 28.5 (C*C*H_2C), 28.7 (C*C*H_2C), 28.8 (C*C*H_2C), 28.9 (C*C*H_2C), 29.0 (d, $J_{C,P}=142.3$, Man-1*), 30.5 (d, $J_{C,P}=6.5$, OCH_2*C*H_2), 33.5 (C*C*H_2C), 46.9 (Et_3N), 60.7 (Man-6), 61.4 (Gal-6), 65.1 (br, O*C*H_2CH$_2$), 69.0 (Gal-4), 69.4 (Man-3), 71.2 (2C, Man-2, br and Gal-2), 72.8 (Gal-3), 73.0 (Man-5), 74.4 (Man-1), 75.7 (Gal-5), 77.3 (Man-4), 103.4 (Gal-1), 114.3 (CH=*C*H_2), 140.7 (*C*H=CH$_2$); ^{31}P NMR (121 MHz, D_2O) δ 22.3; ES-MS (−) data: *m*/*z* 557.03 (100%, $[M-Et_3N-H]^-$) (expected *m*/*z* 557.24, $C_{29}H_{58}NO_{13}P$ requires M, 659.36); $[\alpha]_D$ +12.8 (*c* 1.0, MeOH).