SUPPLEMENTARY INFORMATION

Dissecting the structural and chemical determinants of the 'open-to-closed' motion in the mannosyltransferase PimA from mycobacteria.

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1. SUPPLEMENTARY TABLES

Table S1. Data collection and refinement statistics.

	***Ms*PimA-GMP**
Beamline	Proxima 1
Wavelength (Å)	0.98011
Resolution range (Å)	36.69—2.593 (2.730—2.593)
Space group	$P2_1\ 2_1\ 2_1$
Unit cell	37.32 73.38 138.78 90 90 90
Total reflections	52063 (7702)
Unique reflections	12337 (1783)
Multiplicity	4.2 (4.3)
Completeness (%)	99.01 (99.20)
Mean I/sigma(I)	10.5 (1.7)
Wilson B-factor	53.79
R-sym	0.060 (0.449)
R-factor	0.2028 (0.2825)
R-free	0.2510 (0.3205)
Number of non-H atoms	2851
Macromolecules	2768
Ligands	30
Water	53
Protein residues	367
RMS(bonds)	0.005
RMS(angles)	0.84
Ramach. favored (%)	97
Ramach. outliers (%)	0.27
Clashscore	5.75
Average B-factor	59.90
Macromolecules	60.10
Ligands	78.80
Solvent	48.40

Table S2. Parameters of the unliganded *Ms*PimA, *Ms*PimA•GDP-Man, *Ms*PimA•GDP, *Ms*PimA•GMP, *Ms*PimA•GNO, *Ms*PimA•GUA, *Ms*PimA•Man-PP-Rib, *Ms*PimA•PP-Rib and *Ms*PimA•Man-P complexes from SAXS data.

Data-collection parameters	*Ms*PimA *GDP-Man*	*Ms*PimA *GDP*	*Ms*PimA *GMP*	*Ms*PimA *unliganded*	*Ms*PimA *PP-ribose*	*Ms*PimA *Man-P*	*Ms*PimA *Man-PP-ribose*
Instrument	Beamline B21 (DLS)	Beamline B21 (DLS)	Beamline B21 (DLS)	Beamline B21 (DLS)	Beamline B21 (DLS)	Beamline B21 (DLS)	Beamline B21 (DLS)
Wavelength (Å)	0.9998	0.9998	0.9998	0.9998	0.9998	0.9998	0.9998
q range ($Å^{-1}$) [a]	0.009-0.3700	0.009-0.3700	0.009-0.3700	0.009-0.400	0.009-0.3700	0.009-0.3700	0.009-0.3700
Exposure time (sec)	9	9	9	9	9	9	9
Concentration range (mg ml^{-1})	4 – 8	4 – 8	4 – 8	4 – 8	4 – 8	4 – 8	4 – 8
Temperature (K)	291	291	291	291	291	291	291
Structural parameters[†]							
$I(0)$ (cm^{-1}) [from P(r)]	0.232(4)	0.246(3)	0.237(4)	0.206(2)	0.259(3)	0.248(5)	0.269(5)
R_g (Å) [from P(r)]	29.3(5)	29.2(4)	29.6(3)	30.8(4)	31.1(4)	31.0(5)	31.2(4)
$I(0)$ (cm^{-1}) (from Guinier)	0.2317(3)	0.2462(3)	0.2404(4)	0.2113(3)	0.2651(5)	0.2523(5)	0.2619(7)
R_g (Å) (from Guinier)	29.5(2)	29.4(2)	29.5(2)	30.7(2)	31.2(3)	31.1(3)	31.2(4)
D_{max} (Å)	100	100	100	113	109	108	108
Porod volume estimate ($Å^3$)	92,506	96,619	87,195	83,459	85,480	83,693	87,303
Dry volume calculated from sequence ($Å^3$)	51,165	51,165	51,165	51,165	51,165	51,165	51,165
Molecular-mass determination [b]							
Molecular mass [from Porod V] (kDa)	54(10)	57(10)	51(10)	49(10)	50(10)	49(10)	51(10)
Calculated monomeric from sequence (kDa)	42.3	42.3	42.3	42.3	42.3	42.3	42.3
Software employed							
Primary data reduction	*GDA*	*GDA*	*GDA*	*GDA*	*GDA*	*GDA*	*GDA*
Data processing	*PRIMUS / ScÅtter*	*PRIMUS / ScÅtter*	*PRIMUS / ScÅtter*	*PRIMUS / ScÅtter*	*PRIMUS / ScÅtter*	*PRIMUS / ScÅtter*	*PRIMUS / ScÅtter*

[†] Values in parenthesis are estimated errors approximated to the last decimal place

[a] q-range used for calculation of P(r) function

[b] Molecular mass evaluated with equation $ln\ (Q_R) = k * ln\ (\text{Mass}) + c$, where $Q_R = (V_c^2/R_g)$, and k and c are constants (50).

Table S3. Concentration-dependent variations of zero-angle scattering intensities ($I(0)$) and radii of gyration (R_g).

	$I(0)$ (cm^{-1}) (from Guinier)	R_g (Å) (from Guinier)	$I(0)$ (cm^{-1}) [from P(r)]	R_g (Å) (from Guinier)
***Ms*PimA** (4 mg/mL)	0.1258(2)	29.2(3)	0.127(2)	29.8(5)
*Ms*PimA (6 mg/mL)	0.2113(3)	30.7(2)	0.206(2)	30.8(3)
*Ms*PimA (8 mg/mL)	0.2923(5)	31.(5)	0.284(1)	31(1)
*Ms*PimA *GDP-Man* (4 mg/mL)	0.1679(3)	28.8(5)	0.169(2)	28.8(5)
*Ms*PimA *GDP-Man* (6 mg/mL)	0.2317(3)	29.5(2)	0.232(4)	29.9(5)
*Ms*PimA *GDP-Man* (8 mg/mL)	0.3191(9)	29.5(3)	0.317(2)	29(1)
*Ms*PimA *GDP* (4 mg/mL)	0.1741(2)	29.3(2)	0.174(2)	29.0(4)
*Ms*PimA *GDP* (6 mg/mL)	0.2462(3)	29.4(2)	0.242(3)	29.2(4)
*Ms*PimA *GDP* (8 mg/mL)	0.3209(5)	29.5(2)	0.317(4)	29.4(3)
*Ms*PimA *GMP* (4 mg/mL)	0.1527(2)	28.9(2)	0.153(2)	29.1(5)
*Ms*PimA *GMP* (6 mg/mL)	0.2404(4)	29.5(2)	0.237(2)	29.6(3)
*Ms*PimA *GMP* (8 mg/mL)	0.2937(6)	29.8(3)	0.288(9)	30(1)
*Ms*PimA *PP-ribose* (4 mg/mL)	0.1367(3)	29.7(3)	0.136(2)	29.9(5)
*Ms*PimA *PP-ribose* (6 mg/mL)	0.2651(5)	31.2(3)	0.258(3)	31.1(4)
*Ms*PimA *PP-ribose* (8 mg/mL)	0.2885(5)	31.2(3)	0.282(5)	31.3(4)
MsPimA Man-P (4 mg/mL)	0.1465(2)	29.9(2)	0.145(2)	29.9(5)
MsPimA Man-P (6 mg/mL)	0.252(5)	31.0(3)	0.248(5)	31.0(5)
MsPimA Man-P (8 mg/mL)	0.2864(7)	31.1(3)	0.278(5)	30.8(4)
MsPimA Man-PP-ribose (4 mg/mL)	0.1373(5)	29.5(5)	0.152(3)	30.3(6)
MsPimA Man-PP-ribose (6 mg/mL)	0.2619(7)	31.2(4)	0.269(4)	31.2(4)
MsPimA Man-PP-ribose (8 mg/mL)	0.2879(8)	31.3(4)	0.295(3)	31.3(3)

2. SUPPLEMENTARY FIGURES

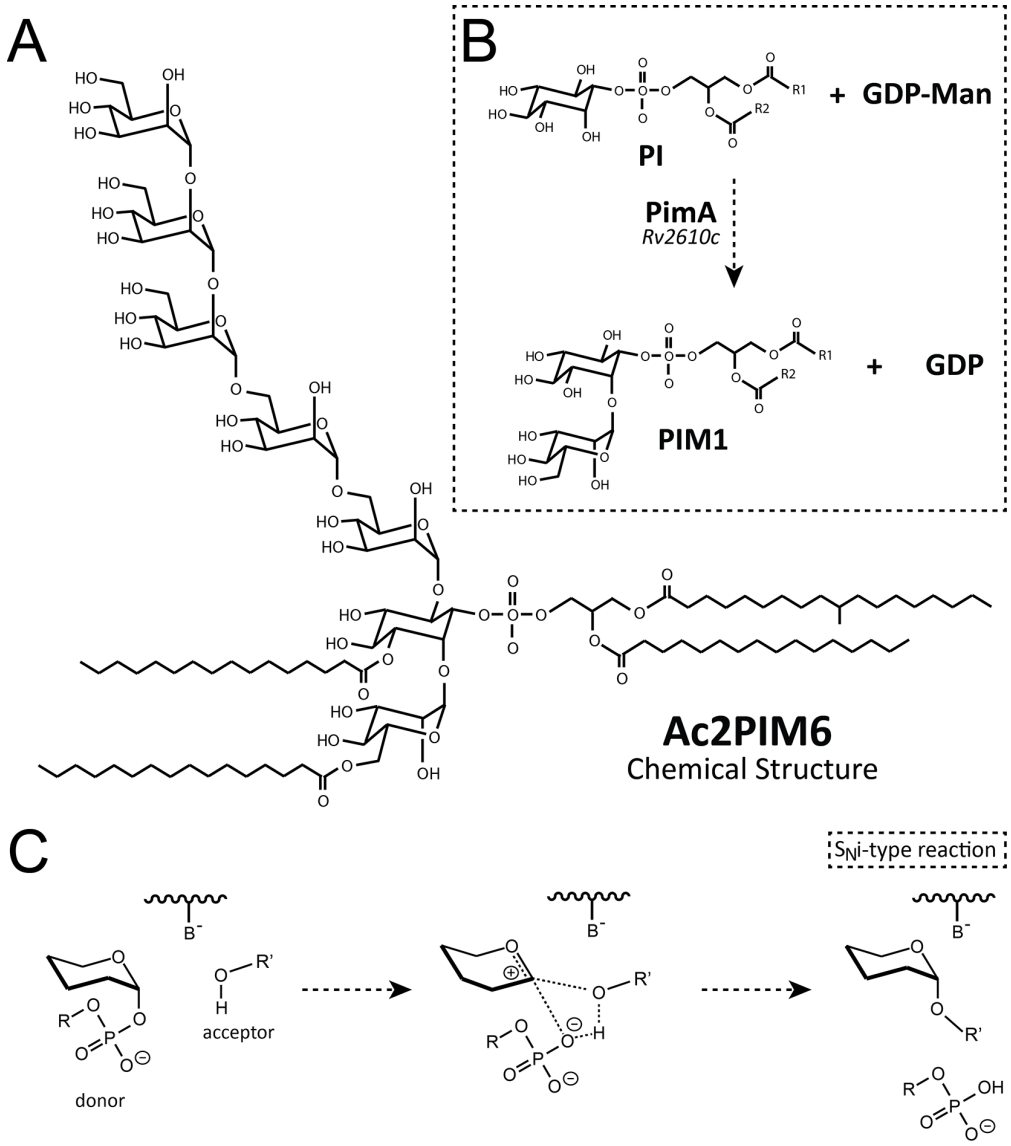


Figure S1. Chemical structure of Ac_2PIM_6 and proposed catalytic mechanism for PimA. *A*. The PIM family of glycolipids comprises PI mono-, di-, tri-, tetra-, penta-, and hexamannosides with different degrees of acylation. PIM_2 and PIM_6 are the two most abundant classes found in *Mycobacterium bovis* bacillus Calmette-Guérin (BCG), *M. tuberculosis* H37Rv, and *Mycobacterium smegmatis* 607 (51). The complete chemical structures of the acylated native forms of PIM_2 and PIM_6 were unequivocally established in *M. bovis* BCG (53, 52). PIM_2 is composed of two Man*p* residues attached to positions 2 and 6 of the *myo*inositol ring of PI. PIM_6 is composed of a pentamannosyl group, *t*–α–Man*p*(1→2)–α–Man*p*(1→2)–α–Man*p*(1→6)–α–Man*p*(1→6)–α–Man*p*(1→, attached to position 6 of the *myo*inositol ring, in addition to the Man*p* residue present at position 2. PIM_2 (53) was found to initially occurs in multiple acylated forms, where two fatty acids are attached to the glycerol moiety, and two additional fatty acids may esterify available hydroxyls on the Man*p* residue and/or the *myo*-inositol ring. The tri- and tetraacylated forms of PIM_2 and PIM_6 (Ac_1PIM_2/Ac_2PIM_2 and Ac_1PIM_6/Ac_2PIM_6) are the most abundant. Ac_1PIM_2 and Ac_1PIM_6 from *M. bovis* BCG show major acyl forms containing two palmitic acid residues (C16) and one tuberculostearic acid residue (10-methyloctadecanoate, C19), where one fatty acyl chain is linked to the Man*p* residue attached to position 2 of *myo*-inositol, and two fatty acyl chains are located on the glycerol moiety. The tetraacylated forms, Ac_2PIM_2 and Ac_2PIM_6, are present predominantly as two populations bearing either three

C16/one C19 or two C16/two C19 (51, 52). Mass spectrometry analyses have led to the conclusion that the glicerol moiety is preferentially acylated with C16/C19. Other acylation positions are C3 of the *myo*-inositol unit and C6 of Man*p* linked to C2 of *myo*-inositol. *B*. PimA catalyzes the transfer of a mannose residue, from GDP-Man, to the 2-position of the inositol ring of PI. *C*. PimA is a retaining glycosyltransferase, proposed to follow an SNi-type reaction mechanism.

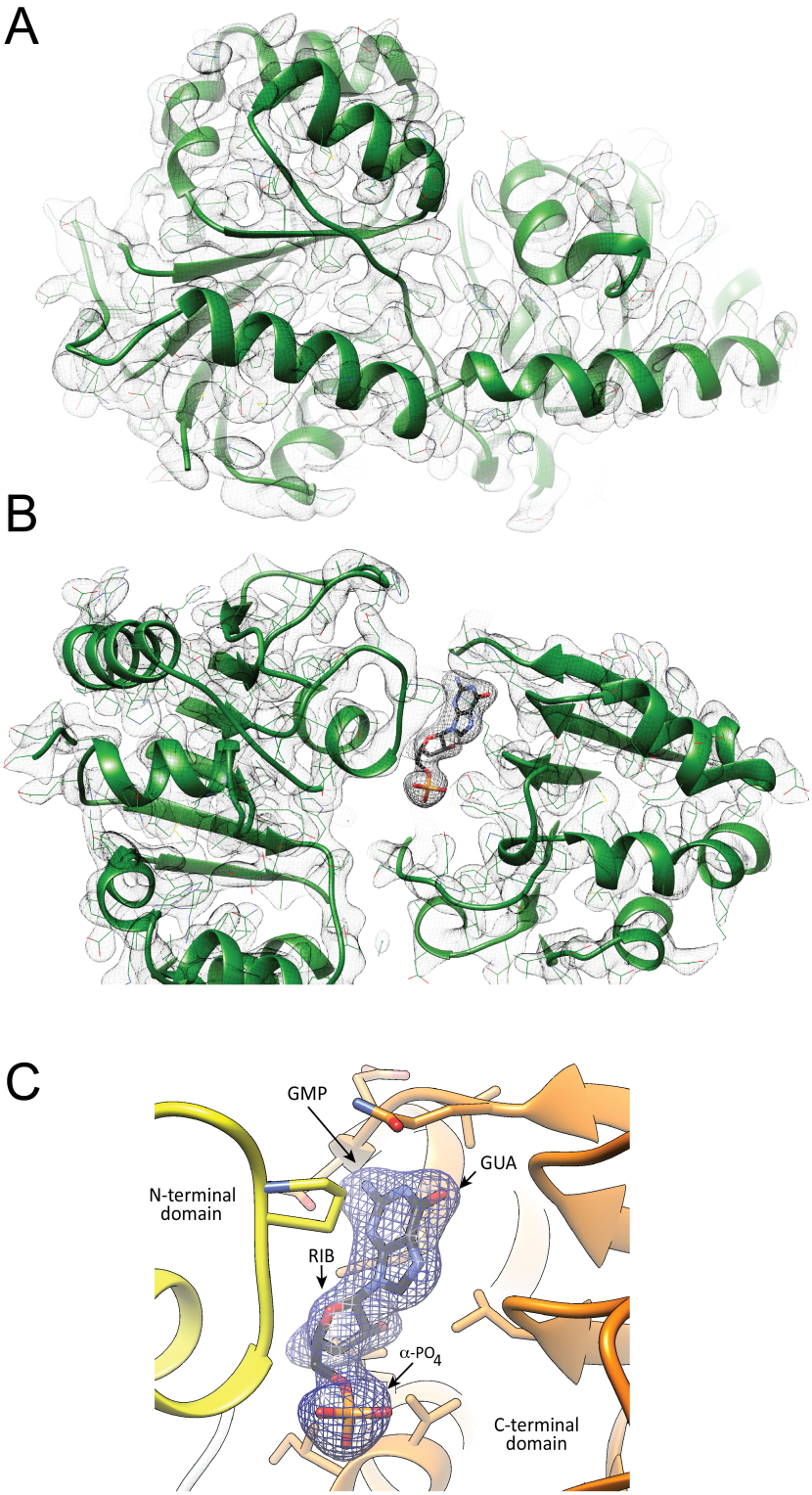
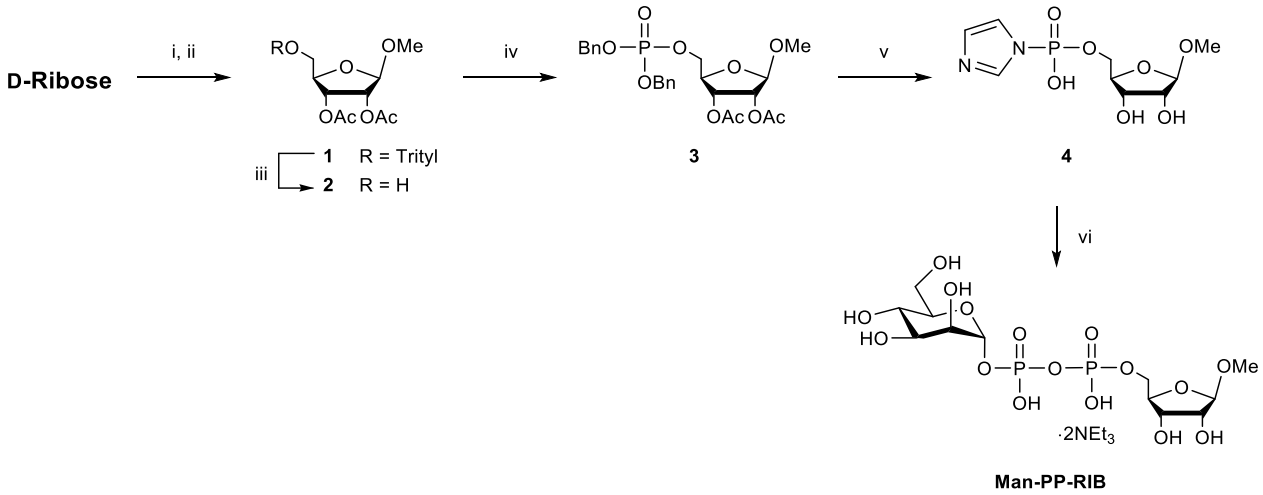


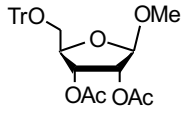
Figure S2. Final (2*Fo-Fc*) electron density map for MsPimA-GMP complex (contoured at 1σ). Final (2*Fo-Fc*) electron density map for the overall structure of the MsPimA-GMP complex (A and B) and GMP (C; contoured at 1σ) is shown.

3. SUPPLEMENTARY MATERIALS AND METHODS

3.1. Chemical synthesis of Man-PP-RIB.

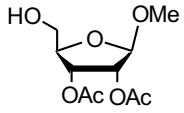


Scheme S1. (i) Dowex 50WX8, MeOH, 18 h, rt, quant. (ii) Trityl chloride, Py, 50 °C, 9 h, then Ac_2O, 15 h, rt, 50%. (iii) AcOH, H_2O, 65 °C, 90 min, 72%. (iv) 1*H*-tetrazole, dibenzyl *N*,*N*-diisopropylphosphoramidite, DCM, 2 h, rt, then ^tBuOOH, 2 h, 0 °C to rt, 49%. (v) H_2, $Pd(OH)_2/C$, NEt_3, MeOH, 24 h, rt. Then, Imidazole, 2,2'-dithiopyridine, PPh_3, NEt_3, DMF, 15 h, rt. 66 %. (vi) α-D-Mannose-1-phosphate, $MgCl_2$, DMF, 24 h, rt. 45%.



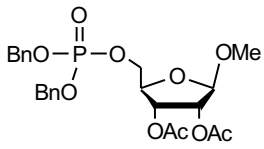
Methyl 2,3-di-*O*-Acetyl-5-*O*-trityl-β-D-ribofuranoside (1). Dowex 50WX8 (2.00 g) was added to a vigorously stirred suspension of D-Ribose (5.00 g, 33.33 mmol), in MeOH (100 mL) and stirred 18 h, at 30 °C. The mixture was filtered over a celite pad, then concentrated under reduced pressure to give the intermediate Methyl D-ribofuranoside (anomeric mixture: 5.50 g, quant.) as a transparent syrup. The residue was dissolved in pyridine (60 mL), treated with trityl chloride (10.2 g, 36.63 mmol) and stirred at 50 °C for 9 h. Then, Ac_2O (20 mL, 211.58 mmol) was added and stirred at rt for 15 h, then concentrated. The residue was dissolved in EtOAc (200 mL) and washed with 1M HCl aqueous solution (3 x 30 mL), then aqueous $NaHCO_3$ sat. solution (30 mL), and brine (30 mL). The organic layer was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (hexanes/EtOAc, 1:0 to 1:1, v/v) to give compound **1** (pure β anomer: 8.20 g, 50 %) as a white foam.

$[\alpha]_D^{21} \cong$ -4 (*c* 1.4, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53-7.46 (m, 6H; Ph); 7.36-7.23 (m, 9H; Ph); 5.41 (dd, J = 6.6, 4.8 Hz, 1H; H-3); 5.29 (dd, J = 4.8, 1.2 Hz, 1H; H-2); 4.95 (d, J = 1.2 Hz, 1H; H-1); 4.30 (ddd, J = 6.6, 5.2, 4.6 Hz, 1H; H-4); 3.37 (s, 3H, OCH_3); 3.31 (dd, J = 10.0, 4.6 Hz, 1H; H-5); 3.25 (dd, J = 10.0, 5.2 Hz, 1H; H-5); 2.12 (s, 3H; Ac); 2.03 (s, 3H; Ac). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.8 (C_{Ac}); 169.7 (C_{Ac}); 143.9 (C_{Ph}); 128.9 (C_{Ph}); 127.9 (C_{Ph}); 127.2 (C_{Ph}); 106.3 (C-1); 86.8 (C_{Tr}); 79.9 (C-4); 74.9 (C-2); 72.2 (C-3); 64.5 (C-5); 55.5 (OCH_3); 20.8 (C_{Ac}); 20.7 (C_{Ac}). Elemental analysis calcd (%) for $C_{29}H_{30}O_7$: C 71.01, H 6.16, O 22.83; found: C 70.95, H 6.07.



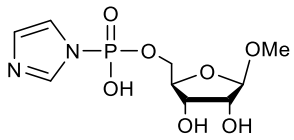
Methyl 2,3-di-*O*-Acetyl-β-D-ribofuranoside (2). A solution of compound **1** (8.20 g, 16.72 mmol) in glacial AcOH (150 mL) was treated with water (40 mL) and stirred for 90 min at 65 °C. The mixture was concentrated under reduced pressure and the residue dissolved in EtOAc (300 mL), washed with aqueous $NaHCO_3$ sat. solution until neutralization, and brine (50 mL). The organic layer was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (hexanes/EtOAc, 1:0 to 1:1, v/v) to give compound **2** (2.97 g, 72 %) as a yellow oil. The physical and spectroscopic properties of the product were found to be consistent to those reported in the literature. (54).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30 (dd, J = 6.1, 5.2 Hz, 1H; H-3); 5.17 (dd, J = 5.2, 1.1 Hz, 1H; H-2) 4.85 (d, J = 1.1 Hz, 1H; H-1); 4.19-4.14 (ddd, J = 6.1, 4.2, 3.4 Hz; 1H; H-4); 3.74 (dd, J = 12.1, 3.4 Hz, 1H; H-5); 3.59 (dd, J = 12.1, 4.2 Hz, 1H; H-5); 3.36 (s, 3H; OCH_3); 2.05 (s, 3H; Ac); 2.00 (s, 3H; Ac).



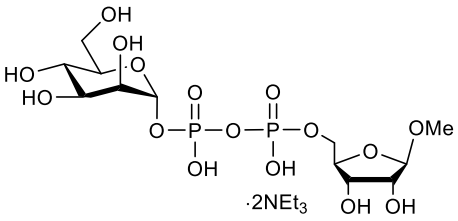
Dibenzyl [1-*O*-methyl-2,3-(di-*O*-acetyl)-5-yl-β-D-ribofuranoside]phosphate (3). To a stirred solution compound **2** (1.00 g, 4.03 mmol) in dry CH_2Cl_2 (50 mL), a 0.45 M solution of 1*H*-Tetrazole in CH_3CN (16.8 mL, 7.55 mmol) and Dibenzyl *N*,*N*-diisopropylphosphoramidite (2.0 mL, 6.04 mmol) were added sequentially via syringe under Ar atmosphere. The reaction mixture was stirred vigorously under inert atmosphere rt for 2 h, at rt. Then, a 5.5 M solution of ^{t}BuOOH in decane (1.1 mL, 6.04 mmol) was added dropwise via syringe to the reaction mixture at 0 °C and stirred 2 h, at rt, then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (DCM/Et_2O, 1:0 to 9:1, v/v) to give compound **3** (1.00 g, 49 %) as a transparent oil.

$[\alpha]_D^{22} \cong$ -10 (*c* 1.3, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41-7.30 (m, 10H; Ph); 5.31 (dd, *J* = 6.8, 5.0 Hz, 1H; H-3); 5.23 (dd, *J* = 5.0, 0.8 Hz, 1H; H-2); 5.07 (dd, *J* = 8.0, 1.2 Hz, 4H; C*H*$_2$Ph); 4.90 (d, *J* = 0.8 Hz, 1H; H-1); 4.33-4.25 (m, 1H; H-4); 4.23-4.01 (m, 2H; H-5); 3.34 (s, 3H; OCH_3); 2.12 (s, 3H; Ac); 2.03 (s, 3H; Ac). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.7 (2 C_{Ac}); 135.9 (C_{Ph}); 135.8 (C_{Ph}); 128.7 (C_{Ph}); 128.7 (C_{Ph}); 128.1 (C_{Ph}); 128.1 (C_{Ph}); 106.4 (C-1); 79.2 (d, *J* = 8.1 Hz; C-4); 74.7 (C-2), 71.5 (C-3); 69.6 (*C*H_2Ph); 69.5 (*C*H_2Ph); 67.9 (d, *J* = 5.6 Hz; C-5), 55.4 (OCH_3); 20.7 (C_{Ac}); 20.6 (C_{Ac}). ^{31}P NMR (121 MHz, $CDCl_3$) δ *-1.21 (s)*. Elemental analysis calcd (%) for $C_{24}H_{29}O_{10}P$: C 56.69, H 5.75, O 31.47, P 6.09; found: C 56.34, H 5.83, P 6.16.



[1-*O*-methyl-β-D-ribofuranoside-5-yl] imidazolyl phosphate (4). Compound **3** (420 mg, 0.82 mmol) is solved in MeOH (5 mL) and NEt_3 (2 mL, 7.5 mmol). $Pd(OH)_2$ (20 % on carbon, 50 mg, 0.07 mmol) was added and atmosphere is replaced by H_2 and purged 3 times prior to hydrogenate at 3 bar for 24 hours at rt. After filtration, solvent was removed under vacuum and the residue was solved in a small quantity of water and freeze-dried. It was resolved in anhydrous DMF (2 mL), with 2,2'-dithiopyridine (540 mg, 2.46 mmol, 3 eq.) and imidazole (557 mg, 8.2 mmol, 10 eq.) and was added trimethylamine (0.45 mL, 3.28 mmol, 4 eq.) and triphenylphosphine (645 mg, 2.46 mmol, 3 eq.) at room temperature under argon. The reaction was stirred for 15 h. The product was precipitated from the reaction mixture by the addition of an anhydrous solution of $NaCLO_4$ (800 mg, 6.5 mmol, 8 eq.) in dry acetone (50 mL). The heterogeneous mixture was cooled at 0ºC under argon. The precipitate was filtered, washed twice with cold, dry acetone and dried *in vacuo* over P_2O_5 to yield **4** (160 mg, 66 %) as a white solid (55).

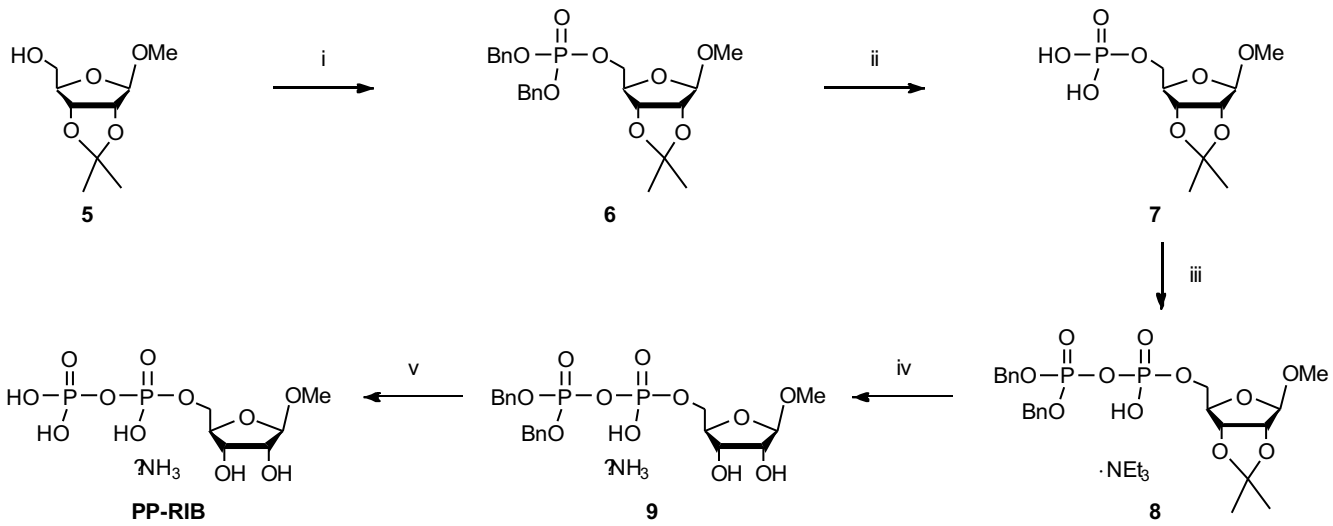
mp: 60-62 °C. $[\alpha]_D^{20} \cong$ -29 (*c* 0.8, H_2O). ^{1}H NMR (400 MHz, D_2O) δ 7.86 (t, J = 1Hz, 1H; H-Im); 7.24 (c, J = 1.5 Hz, 1H; H-Im); 7.06-7.04 (m, 1H; H-Im); 4.78 (d, J = 1 Hz, 1H; H-1); 4.06 (ddd, J = 6.7, 4.7, 0.4 Hz, 1H; H-3); 3.98-3.90 (m, 3H; H-2, H-4, H-5); 3.73 (dt, J = 11.2, 5.4 Hz, 1H; H-5'); 3.20 (s, 3H; OCH_3). ^{13}C NMR (100 MHz, D_2O) δ 136.5 (C_{Im}), 128.4 (C_{Im}); 119.9 (C_{Im}); 107.9 (C-1); 81.4 (C-4); 74.1 (C-2); 70.7 (C-3); 66.5 (C-5); 55.2 (OCH_3). ^{31}P NMR (162 MHz, D_2O) δ -7.80. Elemental analysis calcd (%) for $C_9H_{15}N_2O_7P$: C 36.74; H 5.14; N 9.52; O 38.07; P 10.53; found: C 36.64; H 5. 11; N 9.48; P 10.50.



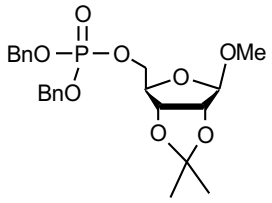
1-*O*-methyl-β-D-ribofuranose-diphosphate-1-α-D-mannose (Man-PP-RIB). α-D-mannose-1-phosphate free acid (52 mg, 0.2 mmol) was dissolved in water (2 mL) and NEt_3, and stirred for 30 minutes. Solvent was removed in vacuo and the resulting syrup was freeze-dried. The lyophilized solid was solved with $MgCl_2$ (38 mg, 0.4 mmol, 2 eq.) in anhydrous DMF (1.5 mL) and a solution of compound **4** (88 mg, 0.3 mmol, 1.5 eq) in anhydrous DMF (1.0 mL) was added under argon. This reaction was stirred for 24 h and the solvent was removed under vacuum. The mixture was purified by semi-preparative HPLC using a Waters Atlantis column eluting with water containing 0.5 % of NEt_3 to afford, after freeze-drying, **Man-PP-RIB** (62 mg, 45 %) as a hygroscopic solid.

$[\alpha]_D^{20} \cong$ -48 (*c* 0.5, H_2O). ^{1}H NMR (500 MHz, D_2O) δ 5.25 (d, J = 7.0 Hz, 1H, H-1_{man}); 4.80 (s, 1H, H-1rib); 4.31 (t, J = 5.6 Hz, 1H, H-3_{rib}); 4.19 (c, J = 6.4 Hz, 1H, H-4_{rib}); 4.09-3.91 (m, 3H, H-2_{rib}, H-5_{rib}); 3.91-3.78 (m, 4H, H-2_{man}, H-4_{man}, H-5_{man}, H-6_{man}); 369-3.62 (m, 1H, H-6'$_{man}$); 3.53 (t, J = 7.7 Hz, 1H, H-3_{man}); 3.32 (s, 3H, O*C*H_3), 2.97 (c, J = 7.3 Hz, 12H, N*C*H_2); 1.27 (t, J = 7.3 Hz, 18H, NCH_2CH_3).^{13}C NMR (125 MHz, D_2O) δ 107.8 (C-1_{rib}); 94.9 (d, J = 5.0 Hz; C-1_{ma}n); 80.9 (d, J = 9.5 Hz; C-4_{rib}); 74.0 (C-2_{rib}); 72.8 (C-4_{man}); 71.1 (d, J = 7.2 Hz; C-2_{ma}n); 70.4 (C-3_{rib}); 70.1 (C-5_{man}); 67.1 (C-3_{man}); 66.6 (d, J = 5.5 Hz; C-5_{rib}); 61.2 (C-6_{man}); 54.9 (O*C*H$_3$): 46.7 (N*C*H$_2$); 8.4 (NCH$_2$*C*H$_3$). ^{31}P NMR (162 MHz, D_2O) δ -11.3 (d, J = 18.8 Hz); -13.8 (d, J = 18.6 Hz). Elemental analysis calcd (%) for $C_{24}H_{54}N_2O_{16}P_2$: C 41.86, H 7.90, N 4.07 O 37.17, P 9.00; found: C 41.63, H 7.98, N 3.95 P 8.91.

3.2. Chemical synthesis PP-RIB.

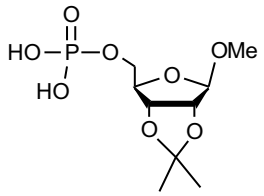


Scheme S2. (i) 1*H*-Tetrazole, Dibenzyl *N*,*N*-diisopropylphosphoramidite, DCM, 2 h, rt, then ^{t}BuOOH, 2 h, 0 °C to rt, 47%**.** (ii) H_2, 35 Bar, $Pd(OH)_2/C$, MeOH, 20 h, rt, quant. (iii) Et_3N, 1*H*-Tetrazole, Dibenzyl *N*,*N*-diisopropylphosphoramidite, DMF, 8 h, rt, then ^{t}BuOOH, 15 h, 0 °C to rt, 68%. (iv) TFA, H_2O, 30 min, rt, 32%. (v) H_2, 30 Bar, $Pd(OH)_2/C$, MeOH/H_2O, NH_4HCO_3 18 h, rt, 86%.



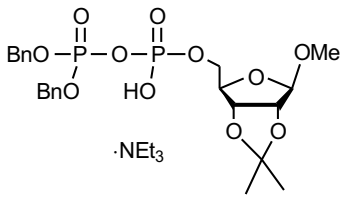
Dibenzyl [1-*O*-methyl-2,3-(di-*O*-isopropylidene)-5-yl-β-D-ribofuranoside]phosphate (6). Compound **5** was prepared according to methods reported in literature (56). The physical and spectroscopic proprieties were found to be identical to those reported. To a stirred solution of compound **5** (500 mg, 2.45 mmol) in dry DCM (30 mL), a 0.45 M solution of 1*H*-Tetrazole in CH_3CN (10.2 mL, 4.59 mmol) and Dibenzyl *N*,*N*-diisopropylphosphoramidite (1.2 mL, 3.67 mmol) were added sequentially via syringe under Ar atmosphere. The reaction mixture was stirred vigorously under inert atmosphere rt for 2 h, at rt. Then, a 5.5 M solution of ^{t}BuOOH in decane (670 µL, 3.75 mmol) was added dropwise via syringe to the reaction mixture at 0 °C and stirred 2 h, at rt, then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (DCM/Et_2O, 1:0 to 97:3, v/v) to give compound **6** (529 mg, 47 %) as a transparent oil.

$[\alpha]_D^{21} \cong$ -31 (*c* 0.9, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.23 (m, 10H; Ph); 4.98 (dd, *J* = 8.4, 3.8 Hz, 4H; C*H*$_2$Ph); 4.86 (s, 1H; H-1); 4.53 (d, *J* = 5.9 Hz, 1H; H-3); 4.44 (d, *J* = 5.9 Hz, 1H; H-2); 4.21 (ddd, *J* = 8.0, 5.9, 1.0 Hz, 1H; H-4); 3.87 (qdd, *J* = 10.3, 8.0, 6.5 Hz, 2H; H-5); 3.18 (s, 3H; OCH_3); 1.39 (s, 3H; CH_{3Isp}); 1.21 (s, 3H; CH_{3Isp}). ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.8 (C_{Ph}); 135.8(C_{Ph}); 128.7(C_{Ph}); 128.1 (C_{Ph}); 112.6 (C_{Isp}); 109.4 (C-1); 85.1 (C-2); 84.7 (d, J = 8.8 Hz; C-4); 81.6 (C-3); 69.6 (*C*H$_2$Ph); 69.6 (*C*H$_2$Ph); 67.2 (d, J = 5.9 Hz; C-5); 55.1 (OCH_3); 26.5 (C_{Isp}); 25.0 (C_{Isp}). ^{31}P NMR (162 MHz, $CDCl_3$) *δ 0.00 (s).* Elemental analysis calcd (%) for $C_{23}H_{29}O_8P$: C 59.48, H 6.29, O 27.56, P 6.67; found: C 60.66, H 6.06, P 6.74.



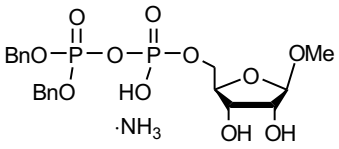
Dihydrogen [1-*O*-methyl-2,3-(di-*O*-isopropylidene)-5-yl-β-D-ribofuranoside]phosphate (7). A stirred solution of compound **6** (529 mg, 1.14 mmol) in MeOH (25 mL), was bubbled with Ar for 15 minutes, and then $Pd(OH)_2$ (20 % on carbon, 80 mg, 0.14 mmol) was added under inert atmosphere. The Ar atmosphere was substituted with H_2 and purged 5 times prior to hydrogenate at 35 bar for 20 hours at rt. The mixture was filtered over a celite pad, and then concentrated under reduced pressure to give **7** (323 mg, quant.) as a transparent liquid, used in the next step without further purification.

$[\alpha]_D^{24} \cong$ -46 (*c* 1.2, MeOH). ^{1}H NMR (300 MHz, Methanol-d_4) δ 4.94 (s, 1H; H-1); 4.78 (d, J = 6.0 Hz, 1H; H-3); 4.62 (d, J = 6.0 Hz, 1H; H-2); 4.33-4.26 (m, 1H; H-4), 3.99-3.87 (m, 2H; H-5); 3.34 (s, 3H; OCH_3); 1.45 (s, 3H; CH_3), 1.32 (s, 3H; CH_3). ^{13}C NMR (75 MHz, Methanol-d_4) δ 113.5 (C_{Isp}); 110.7 (C-1); 86.3 (d, J = 9.1 Hz; C-4); 86.3 (C-2); 82.9 (C-3); 67.5 (d, J = 5.5 Hz; C-5); 55.3 (OCH_3); 26.7 (C_{Isp}); 25.0 (C_{Isp}). ^{31}P NMR (121 MHz, Methanol-d_4) *δ 0.13 (br)*. Elemental analysis calcd (%) for $C_9H_{17}O_8P$: C 38.04, H 6.03, O 45.04, P 10.90; found: C 38.69, H 6.19, P 10.76.



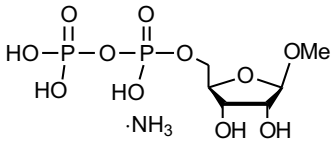
Dibenzyl [1-*O*-methyl-2,3-(di-*O*-isopropylidene)-5-yl-β-D-ribofuranoside] triethylammonium diphosphate (8). Compound **7** (320 mg, 1.13 mmol) was dissolved in dry DMF and concentrated under reduced pressure 3 times prior to use. The resulting dry residue was dissolved in anhydrous DMF (40 mL), and to the stirred solution, dry Et_3N (155 µL, 1.13 mmol) and then a 0.45 M solution of 1*H*-Tetrazole in CH_3CN (6.2 mL, 2.79 mmol) were added sequentially via syringe under Ar atmosphere. To the resulting mixture Dibenzyl *N*,*N*-diisopropylphosphoramidite (760 µL, 2.25 mmol) was added via syringe and stirred vigorously under inert atmosphere for 8 h, at rt. Then, a solution of ^{t}BuOOH in decane (400 µL, 2.25 mmol) was added dropwise to the reaction mixture at 0 °C and stirred 15 h at rt, then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (DCM/MeOH, 1:0 to 95:5 containing 1 % Et_3N, v/v) to give compound **8** (493 mg, 68 %) as a transparent syrup.

$[\alpha]_D^{24} \cong$ -28 (*c* 1.5, MeOH). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.43-7.32 (m, 10H; Ph); 5.18-5.12 (m, 4H; CH_2Ph); 4.90 (s, 1H; H-1); 4.81 (d, J = 6.0 Hz, 1H; H-3); 4.57 (d, J = 6.0 Hz, 1H; H-2); 4.31 (dd, J = 8.8, 6.0 Hz, 1H; H-4); 4.01-3.92 (m, 2H; H-5), 3.28 (s, 3H; OCH_3); 3.17 (q, J = 7.3 Hz, 6H; NCH_2CH$_3$) 1.43 (s, 3H; CH_{3Isp}); 1.29 (t, J = 7.3 Hz, 9H; NCH$_2$CH_3); 1.28 (s, 3H; CH_{3Isp}). ^{13}C NMR (75 MHz, Methanol-d_4) δ 136.0 (C_{Ph}); 135.9 (C_{Ph}); 128.2 (C_{Ph}); 128.2 (C_{Ph}); 127.7 (C_{Ph}); 127.7 (C_{Ph}); 112.0 (C_{Isp}); 109.3 (C-1); 85.1 (d, J = 9.6 Hz; C-4); 85.0 (C-2); 81.7 (C-3); 69.5 (d, J = 5.7 Hz; *C*H$_2$Ph); 69.5 (d, J = 5.7 Hz; *C*H$_2$Ph); 66.4 (d, J = 6.3 Hz; C-5); 53.8 (OCH_3); 46.3 (N*C*H$_2$CH$_3$); 24.3 (C_{Isp}); 23.6 (C_{Isp}); 7.8 (NCH$_2$*C*H$_3$). ^{31}P NMR (162 MHz, Methanol-d_4) δ -11.96 (d, J = 20.0 Hz); -12.69 (d, J = 20.0 Hz). Elemental analysis calcd (%) for $C_{29}H_{45}NO_{11}P_2$: C 53.95, H 7.03, N 2.17, O 27.26, P 9.60; found: C 53.04, H 6.83, N 2.25, P 9.74.



Dibenzyl [1-*O*-methyl-5-yl-β-D-ribofuranoside] ammonium diphosphate (9). Compound **8** (250 mg, 0.39 mmol) was suspended in H_2O (7 mL), treated with TFA (7 mL) and stirred at rt for 30 min, then concentrated under reduced pressure. The residue was eluted trough Dowex 50W X8 (NH_4^+ form) with H_2O/MeOH (70:30, v/v) solution. The elutant was concentrated under reduced pressure and the residue purified on a Sephadex LH-20 (1 x 70 cm) column, eluted with 100 mM NH_4HCO_3 in H_2O/MeOH (70:30, v/v) solution and concentrated under reduced pressure to give **9** (65 mg, 32 %) as a transparent syrup.

$[\alpha]_D^{22} \cong$ -10 (*c* 1.0, MeOH). ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.41-7.25 (m, 10H; Ph); 5.14 (d, J = 8.0 Hz, 4H; CH_2Ph); 4.74 (d, J = 0.9 Hz, 1H; H-1); 4.18-3.96 (m, 4H; H-3, H-4, H-5); 3.89 (dd, J = 4.6, 0.9 Hz, 1H; H-2); 3.30 (s, 3H; OCH_3). ^{13}C NMR (75 MHz, Methanol-d_4) δ 137.4 (C_{Ph}); 137.3 (C_{Ph}); 129.5 (C_{Ph}); 129.5 (C_{Ph}); 129.1 (C_{Ph}); 109.8 (C-1); 83.1 (d, J = 9.2 Hz; C-4); 76.2 (C-2); 77.9 (C-3); 70.9 (*C*H_2Ph); 70.9 (*C*H_2Ph); 69.1 (d, J = 6.0 Hz; C-5); 55.3 (OCH_3). ^{31}P NMR (121 MHz, Methanol-d_4) δ -11.51 (d, J = 19.7 Hz); -12.77 (d, J = 19.7 Hz). Elemental analysis calcd (%) for $C_{20}H_{29}NO_{11}P_2$: C 46.07, H 5.61, N 2.69, O 33.75, P 11.88; found: C 45.89, H 5.45, N 2.66, P 11.67.



Dihydrogen [1-*O*-methyl-5-yl-β-D-ribofuranoside] ammonium diphosphate (PP-RIB). To a stirred solution of **9** (31 mg, 0.06 mmol) in MeOH (3 mL), H_2O (1 mL) and NH_4HCO_3 (24 mg, 0.30 mmol) were added. The resulting solution was then bubbled with Ar for 15 minutes, and then $Pd(OH)_2$ (20 % on carbon, 21 mg, 0.03 mmol) was added under inert atmosphere. The Ar atmosphere was substituted with H_2 and purged 5 times prior to hydrogenate at 30 bar for 18 hours at rt. The mixture was filtered over a celite pad, and then concentrated under reduced pressure. The residue was purified on a Sephadex LH-20 (1 x 70 cm) column, eluted with 100 mM NH_4HCO_3 in H_2O solution and concentrated under reduced pressure to give **PP-RIB** (18 mg, 86 %) as a white solid.

mp: 73-75 °C. $[\alpha]_D^{22} \cong$ -13 (*c* 0.9, H_2O). ^{1}H NMR (500 MHz, $D_2O^{25°C}$) δ 4.90 (d, J = 1.3 Hz, 1H; H-1); 4.29 (dd, J = 6.7, 4.7 Hz, 1H; H-3); 4.13 (dd, J = 6.5, 3.8 Hz, 1H; H-4); 4.11-4.04 (m, 2H; H-2, H-5); 4.00 (dd, J = 11.0, 5.6 Hz, 1H; H-5); 3.40 (s, 3H; OCH_3). ^{13}C NMR (125 MHz, $D_2O^{25°C}$) δ 107.9 (C-1); 81.3 (d, J = 7.9 Hz; C-4); 74.0 (C-2); 70.5 (C-3); 65.9 (d, J = 3.7 Hz; C-5); 55.3 (OCH_3). ^{31}P NMR (121 MHz, $D_2O^{25°C}$) δ -6.30 (br); -10.35 (br). Elemental analysis calcd (%) for $C_6H_{17}NO_{11}P_2$: C 21.12, H 5.02, N 4.11, O 51.59, P 18.16; found: C 21.24, H 4.89, N 4.18, P 18.88.

3.3. ^{1}H and ^{13}C NMR spectra of Man-PP-RIB, PP-RIB and precursors compounds.

4. SUPPLEMENTARY REFERENCES

50. Rambo RP, Tainer JA. (2013). Accurate assessment of mass, models and resolution by small-angle scattering. *Nature*. 496, 477-481.
51. Gilleron M, Ronet C, Mempel M, Monsarrat B, Gachelin G, Puzo G. (2001). Acylation state of the phosphatidylinositol mannosides from *Mycobacterium bovis* bacillus Calmette Guérin and ability to induce granuloma and recruit natural killer T cells. *J. Biol. Chem.* 276, 34896–34904.
52. Gilleron M, Quesniaux VF, Puzo G. (2003). Acylation state of the phosphatidylinositol hexamannosides from *Mycobacterium bovis* bacillus Calmette Guerin and *Mycobacterium tuberculosis* H37Rv and its implication in Toll-like receptor response. *J. Biol. Chem.* 278, 29880–29889.
53. Brennan P, Ballou CE. (1967). Biosynthesis of mannophosphoinositides by *Mycobacterium phlei*. The family of dimannophosphoinositides. *J. Biol. Chem.* 242, 3046–3056.
54. Taverna-Porroa M, Bouvier LA, Pereira CA, Montserrat JM, Iribarren AM. 2008. Chemoenzymatic preparation of nucleosides from furanoses. *Tetrahedron Lett.* 49, 2642–2645.
55. Li T, Tikad A, Pan W, Vincent SP. 2014. β-Stereoselective phosphorylations applied to the synthesis of ADP- and polyprenyl-β-mannopyranosides. *Org. Lett.* 16, 5628–5631.
56. Van derpoorten K, Migaud ME. 2004. Isopolar phosphonate analogue of adenosine diphosphate ribose. *Org. Lett*. 6, 3461–3464.