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Synthesis of methyl β-D-arabinofuranoside 5-[1D (and L)-*myo*-inositol 1-phosphate], the capping motif of the lipoarabinomannan of *Mycobacterium smegmatis*

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Abstract

The total synthesis of methyl β-D-arabinofuranoside 5-(*myo*-inositol 1-phosphate), the capping motif of the lipoarabinomannan (LAM) of *Mycobacterium smegmatis*, has been completed. The stereoselective synthesis of β-D-arabinofuranosides has been achieved via an internal aglycon delivery approach using Ogawa and Ito's method. Coupling with enantiomeric *myo*-inositol derivatives gave the diastereoisomeric title compounds in good overall yield. Comparison with the natural product firmly established the proposed structure for the capping of the LAM but left the absolute configuration of the *myo*-inosityl moiety undetermined.

Keywords: *Mycobacterium smegmatis*; Lipoarabinomannan; β-D-Arabinofuranoside; Sugar phosphates

1. Introduction

Lipoarabinomannans (LAMs) are highly antigenic oligosaccharide components, ubiquitous to the *Mycobacterium* genus, which play a key role in the immunopathogenicity of these microorganisms [1]. They are isolated from the cell wall and share common structural features: a phosphatidyl-*myo*-inositol anchor and an oligomannan core substituted by an arabinan domain [1]. The nonreducing ends of this arabinan moiety are terminated by β-D-arabinofuranosidic units substituted at C-5 by small motifs, highly variable on the mycobacterial species [2–5]. Strong modulations of the biological properties of the LAMs isolated from various species of mycobacteria were observed and these variations have been tentatively correlated to small structural differences. In the LAM isolated from the nonpathogenic species *Mycobacterium smegmatis* [5], the hydroxymethyl group of the terminal β-D-arabinofuranosides is esterified by a phosphate group carrying a 1-*myo*-inositol of undetermined series. This LAM was found to be an activator of TNF-α production by human macrophages (THP-1 cell line) at a concentration of 10 μg/mL and this activity was reduced after a mild basic treatment [5]. On the other hand, LAMs isolated from *M. tuberculosis* and *M. bovis* BCG were devoid of this activity; in these compounds, the caps on the terminal arabinofuranosides are small α-(1→2)-linked oligomannosides [2,3].

We now report the synthesis of methyl β-D-arabinofuranoside 5-[1D (and L)-*myo*-inositol

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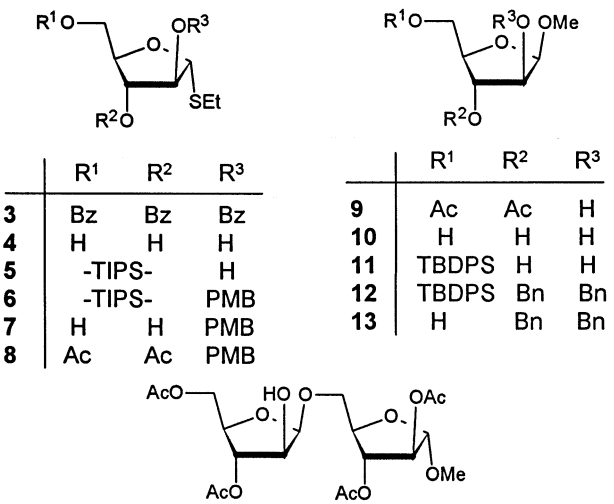
1-phosphate], **1** and **2**, the capping motif of the lipoarabinomanan of *M. smegmatis*. We also report the use of the internal aglycon delivery approach described by Ogawa et al. [6,7] for the first stereoselective synthesis of β-D-arabinofuranosides [8], compounds of interest as potential arabinosyl transferase inhibitors [9–11].

2. Results and discussion

Synthesis of β-D-arabinofuranosides.— Among the numerous methods of intramolecular glycosylation recently described for the stereoselective synthesis of β-mannosides [12–16], Ogawa's procedure [6,7], which uses the *p*-methoxybenzylidene group as temporary linker, was found to be the most convenient [17,18] (Scheme 1).

Treatment of methyl tri-*O*-benzoyl-α-D-arabinofuranoside [19] with ethanethiol and boron trifluoride–etherate gave the ethyl thioglycoside **3** in 80% yield. The benzoyl protecting groups were removed with a catalytic amount of sodium methoxide and simultaneous protection of the 3 and 5 positions of triol **4** was achieved with 1 equivalent of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPS chloride) in pyridine to give **5** in 75% yield [20–22]. Introduction of the *p*-methoxybenzyl ether at C-2 of **5** was somewhat troublesome and various benzylation procedures (*p*MeOBnCl, NaH, DMF or *p*MeOBnCl, Bu_4NHSO_4, CH_2Cl_2–NaOH [23]) were ineffective. This hydroxyl group was eventually benzylated in 59% yield with *p*-methoxybenzyl bromide [24] and 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP) [25–27] in acetonitrile. Compound **6** was first treated with 1.5 equivalent of 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) and 1 equivalent of methanol and the very sensitive intermediate mixed acetal [6,7] was then activated with iodonium dicollidine perchlorate (IDCP) [28] in the presence of a catalytic amount of trimethylsilyltriflate (Me_3SiOTf) to promote the glycosylation [29,30]. Under these conditions, the TIPS protecting group was lost and only degradation products were observed. The TIPS group in **6** was thus removed and replaced by acetyl groups through successive treatment of **6** with tetrabutylammonium fluoride (nBu_4NF) in tetrahydrofuran (THF) and acetylation with acetic anhydride in pyridine to **8** (77%, two steps). Compound **8** was then sequentially treated as above with DDQ–methanol and IDCP–Me_3SiOTf to give anomerically pure methyl 3,5-di-*O*-acetyl-β-D-arabinofuranoside (**9**) in 51% yield from **8**. The β-(D) configuration of **9** was established from the ^1H and ^{13}C NMR data ($\delta_{H\text{-}1}$ 4.90 ppm, $J_{H\text{-}1,H\text{-}2}$ 4.5 Hz, $\delta_{C\text{-}1}$ 102.3 ppm) [31–34]; deacetylation then furnished methyl β-D-arabinofuranoside (**10**) in 80% yield [35].



	R1	R2	R3
3	Bz	Bz	Bz
4	H	H	H
5	-TIPS-		H
6	-TIPS-		PMB
7	H	H	PMB
8	Ac	Ac	PMB

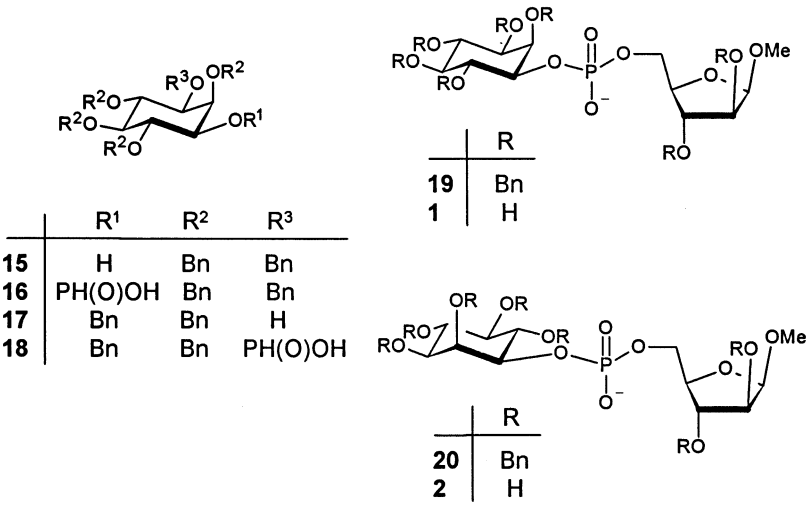
	R1	R2	R3
9	Ac	Ac	H
10	H	H	H
11	TBDPS	H	H
12	TBDPS	Bn	Bn
13	H	Bn	Bn

14

Scheme 1.

In the same manner, the β-linked diarabinofuranoside **14** was obtained in two steps from **8**. Reaction of **8** with 1.5 equivalents of DDQ and 1.05 equivalents of methyl 2,3-di-*O*-acetyl-α-D-arabinofuranoside [36] gave the intermediate acetal in 71% yield after purification on silica, intramolecular glycosylation as above gave disaccharide **14** with the β-(D) configuration at the new anomeric center as the only isolated coupling product (74%, $\delta_{H\text{-}1'}$ 5.12 ppm, $J_{H\text{-}1',H\text{-}2'}$, 4.5 Hz, $\delta_{C\text{-}1'}$ 101.6 ppm) [31–34].

*Synthesis of 5-(*myo*-inositol 1-phosphate) β-D-arabinofuranosides.*—The primary hydroxyl group of methyl β-D-arabinofuranoside (**10**) was selectively silylated with 1 equivalent of *tert*-butyldiphenylsilyl chloride in dimethylformamide (65%) and the 2 and 3 positions of



	R^1	R^2	R^3
15	H	Bn	Bn
16	PH(O)OH	Bn	Bn
17	Bn	Bn	H
18	Bn	Bn	PH(O)OH

	R
19	Bn
1	H

	R
20	Bn
2	H

Scheme 2.

11 were then benzylated (BnBr, NaH) to give **12** in 87% yield. Deprotection of the *tert*-butyldiphenylsilyl group with nBu$_4$NF in THF afforded the 2,3-diprotected arabinofuranoside acceptor **13** in 74% yield.

Enantiomeric 1L- and 1D-1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol (**15** and **17**) were obtained from the racemic compound by resolution with (1*S*)-camphanic acid chloride [37,38], and each enantiomer was then carried out along the synthesis. The 1L-1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol (**15**) {$[\alpha]_D^{25}$ $-9.8°$, (*c* 1.08, $CHCl_3$), lit. $-9.0°$ [37]} was converted to the H-phosphonate **16** by treatment with 3 equivalents of phosphorus trichloride and imidazole [39–41] in 57% yield {$[\alpha]_D^{25}$ $+22°$ (*c* 1.49, $CHCl_3$)}. Activation of the H-phosphonate **16** with 3 equivalents of pivaloyl chloride in pyridine and coupling with **13** in the presence of 4 Å molecular sieves gave the phosphodiester **19** after oxidation with iodine (67% yield, two steps). Final deprotection of the benzyl groups was achieved by hydrogenolysis with palladium hydroxide on carbon and gave compound **1** in 79% yield (Scheme 2).

A similar sequence was carried out from **17** {$[\alpha]_D^{25}$ $+9.7°$ (*c* 1.00, $CHCl_3$), lit. $+9.2°$ [37]}. Conversion to the H-phosphonate **18** {56%, $[\alpha]_D^{25}$ $-23°$ (*c* 1.65, $CHCl_3$)}, coupling with **13** under pivaloyl chloride activation followed by iodine oxidation to **20** (69%, two steps) and hydrogenolysis of the benzyl groups gave the phosphodiester **2**.

The ^{1}H, ^{13}C and ^{31}P NMR spectra for the two diastereoisomers **1** and **2** were then recorded and compared with the reported values for the LAM of *M. smegmatis* [5]. NMR data for **1** and **2** were found to be very similar and very close to the data for the natural product (see Table 3), only minor differences could be observed. Furthermore, in the ^{1}H NMR spectrum of the natural product, a severe crowding in the region of the sugar protons was observed, which precluded complete assignment of the signals of the arabinofuranoside moiety. Although these data unambiguously confirmed the proposed structure for the capping motif of the LAM of *M. smegmatis*, no correlation could be drawn with the absolute configuration of the *myo*-inositol moiety in the natural product, which then remains undetermined.

No TNF-α production by macrophages (cell line THP-1) could be detected with compounds **1** and **2** up to a concentration of 100 μg/mL. Finally, compounds **1** and **2** were shown to be stable to the basic conditions used on the natural product (NaOH, 0.1 N, 40 °C, 2 h). This result indicates that the observed diminution of TNF-α production by macrophages after basic treatment of the natural LAM was not caused by some hydrolysis of the inositol phosphate [5].

3. Conclusions

The internal aglycon delivery approach developed by Ogawa et al. [6,7] was found to offer a general and stereospecific access to β-D-arabinofuranoside compounds. This was used for the synthesis of methyl β-D-arabinofuranoside 5-(*myo*-inositol 1-phosphate), the proposed structure for the caps of the LAMs isolated from *M. smegmatis* which was fully confirmed. In order to ascertain the absolute configuration of the *myo*-inositol in these caps, derivatization of compounds **1** and **2** for comparison with fragments obtained from the degradation of the LAM of *M. smegmatis* are currently under progress and will be reported in the future.

4. Experimental

General methods.—Reactions were performed under argon in anhyd purified solvents. NMR spectra were recorded on Bruker DPX 250, AMX 250 (operating at 250 MHz for ^{1}H and at 62.9 MHz for ^{13}C) and DMX 500 (operating at 500 MHz for ^{1}H, at 125.8 MHz for ^{13}C, and at 202.46 MHz for ^{31}P) spectrometers, chemical shifts are expressed in ppm, from Me_4Si for ^{1}H and ^{13}C spectra and from H_3PO_4 for ^{31}P spectra. Optical rotations were recorded on a Perkin–Elmer 41 polarimeter. Mass spectra were obtained on a Perkin–Elmer SCIEX API 300 (ion spray, IS) or Finnigan MAT TSQ 700 (electrospray, negative mode, ES). Elemental analyses were carried out at the Service Central de Microanalyse du CNRS at Vernaison or at the 'Laboratoire de Chimie de Coordination du CNRS' at Toulouse.

Ethyl 2,3,5-tri-O-*benzoyl-1-thio*-α-D-*arabinofuranoside* (**3**).—Methyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside [19] (12.0 g, 25 mmol) in anhyd CH_2Cl_2, EtSH (2.80 mL, 38 mmol) and $BF_3{\cdot}Et_2O$ (3.83 mL, 30 mmol) were kept for 16 h at room temperature (rt), then neutralized with triethylamine and concentrated. Chromatography (4:1 petroleum ether–EtOAc) gave **3** as a colorless oil (10.35 g, 81%); $[\alpha]_D^{25}$ +22.5° (*c* 1.29, $CHCl_3$); ^{1}H NMR data see Table 1; ^{13}C NMR ($CDCl_3$): δ 166.05, 165.50, 165.33 (C=O), 133.50, 133.44, 132.97, 129.93, 129.87, 129.75, 129.62, 129.56, 128.85, 128.40, 128.21, 127.80 (Ar), 88.05 (C-1), 82.90, 80.60, 78.03 (C-2, C-3, C-4), 63.35 (C-5), 25.21 (CH_2S), 14.80 (CH_3CH_2S). Anal. Calcd for $C_{28}H_{26}O_7S$: C, 66.39; H, 5.17. Found: C, 66.21; H, 5.28.

Ethyl 3,5-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-1-thio*-α-D-*arabinofuranoside* (**5**).—Compound **3** (8.0 g, 16 mmol) was treated at rt with MeONa in MeOH (0.1 M, 30 mL). After completion of the reaction, the mixture was neutralized with Amberlyst IR

Table 1
^{1}H NMR chemical shifts (δ), apparent multiplicities and coupling constants in Hz for compounds **3**, **5**, **6** and **8**

H	**3**	**5**	**6**	**8**
1	5.64 d $J_{1,2}$ 1.2	5.09 d $J_{1,2}$ 5.5	5.21 d $J_{1,2}$ 4.2	5.37 d $J_{1,2}$ 2.2
2	5.56 dd $J_{2,3}$ 1.2	4.10 dd $J_{2,3}$ 6.5	3.88 dd $J_{2,3}$ 5.8	3.94 t $J_{2,3}$ 2.2
3	5.62 dd $J_{3,4}$ 2.5	4.20 dd $J_{3,4}$ 8.2	4.27 dd $J_{3,4}$ 7.2	5.08 dd $J_{3,4}$ 4.0
4	4.79 m	3.89 ddd *J* 3.0, 6.2	3.91 m	4.31 m
5a	4.79 m	3.97 m	3.97 m	4.31 m
5b	4.79 m	3.97 m	3.97 m	4.31 m
Other signals	CH_3 1.36 t *J* 7.5 CH_2 2.77 m	CH_3 1.29 t *J* 7.5 CH_2 2.77 m OH 2.19 m	CH_3 1.29 t *J* 7.5 CH_2 2.69 m OCH_2Ph 4.58 2d	CH_3 1.30 t *J* 7.5 OAc 2.06; 2.09, s CH_2 2.68 m OCH_2Ph 4.55 2d

120 resin (H^+), filtered and the solvent evaporated. Filtration on silica (10:1 CH_2Cl_2–MeOH) gave ethyl 1-thio-α-D-arabinofuranoside (**4**) (2.09 g). This compound was taken up in pyridine (30 mL) and treated with TIPS chloride (4 mL, 12.50 mmol) at rt. After 3 h at rt, the solvent was evaporated and the last traces of pyridine were removed by coevaporation with toluene. The residue was taken up in EtOAc and washed once with water before drying on $MgSO_4$. Chromatography (3:1 petroleum ether–EtOAc) gave **5** as a colorless oil (4.50 g, 65%); $[\alpha]_D^{25}$ +71° (*c* 1.26, $CHCl_3$); ^1H NMR data see Table 1; ^{13}C NMR ($CDCl_3$): δ 87.99 (C-1), 82.08 (C-2), 80.04 (C-4), 76.23 (C-3), 61.04 (C-5), 25.56 (*C*H_2S), 17.41, 17.29, 17.07, 17.04, 16.97 (*i*Pr), 15.02 (*C*H_3CH$_2$S), 13.45, 13.09, 12.74, 12.51 (*i*Pr); ISMS: *m*/*z* 454 [M − 1 + Na]. Anal. Calcd for $C_{19}H_{40}O_5SSi_2$: C, 52.25; H, 9.23. Found: C, 51.81; H, 9.18.

*Ethyl 2-*O*-(4-methoxybenzyl)-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-1-thio-α-*D*-arabinofuranoside* (**6**).—A solution of **5** (3.75 g, 8.58 mmol) in MeCN (25 mL) was cooled to 0 °C before addition of 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (5 mL, 17.27 mmol) and a solution of 4-methoxybenzyl bromide (9.10 g, 42.30 mmol) in 10 mL of MeCN. The reaction medium was kept for 15 min at 0 °C and then 4 h at rt before concentration. The residue was extracted with EtOAc–satd $NaHCO_3$ and the organic phase was dried on $MgSO_4$. Chromatography (25:1 petroleum ether–EtOAc) gave **6** as a slightly yellow oil (2.26 g, 59%); $[\alpha]_D^{25}$ +60° (*c* 1.01, $CHCl_3$); ^1H NMR data see Table 1; ^{13}C NMR ($CDCl_3$): δ 159.21, 129.87, 129.41, 113.67 (Ar), 89.07 (C-2), 86.42 (C-1), 79.67 (C-4), 76.05 (C-3), 72.30 (O*C*H_2Ar), 61.11 (C-5), 55.26 (O*C*H_3), 25.46 (*C*H_2S), 17.44, 17.32, 17.09, 17.05, 16.98 (*i*Pr), 14.91 (*C*H_3CH$_2$S), 13.49, 13.11, 12.82, 12.50 (*i*Pr); ISMS: *m*/*z* 557 [M + 1]. Anal. Calcd for $C_{27}H_{48}O_6SSi_2$: C, 58.23; H, 8.69. Found: C, 58.39; H, 8.66.

*Ethyl 3,5-di-*O*-acetyl-2-*O*-(4-methoxybenzyl)-1-thio-α-*D*-arabinofuranoside* (**8**).—A solution of **6** (1.75 g, 3.14 mmol) in dry THF (25 mL) was treated with nBu$_4$NF (1 M in THF, 6.3 mL, 6.3 mmol) for 15 min at rt. After evaporation of the solvent, the crude product was dried under high vacuum and taken up in pyridine (2 mL) and acetic anhydride (2 mL) for 4 h. Chromatography (3:1 petroleum ether–EtOAc) gave **8** as a slightly yellow oil (965 mg, 77%); $[\alpha]_D^{25}$ +113° (*c* 1.05, $CHCl_3$); ^1H NMR data see Table 1; ^{13}C NMR ($CDCl_3$): δ 170.73, 170.13 (C=O), 159.42, 129.50, 129.21, 113.84 (Ar), 88.00 (C-1), 87.49 (C-2), 79.68 (C-4), 77.72 (C-3), 71.70 (O*C*H_2Ar), 63.13 (C-5), 55.27 (O*C*H_3), 25.42 (*C*H_2S), 20.91, 20.80 (*C*H_3CO), 14.85 (*C*H_3CH$_2$S); ISMS: *m*/*z* 420 [M − 1 + Na]. Anal. Calcd for $C_{19}H_{26}O_7S$: C, 57.26; H, 6.58. Found: C, 57.44; H, 6.61.

*Methyl 3,5-di-*O*-acetyl-β-*D*-arabinofuranoside* (**9**).—A solution of **8** (650 mg, 1.63 mmol), MeOH (80 μL, 1.97 mmol) in CH_2Cl_2 (30 mL) was treated with activated 3 Å molecular sieves for 20 min before addition of DDQ (555 mg, 2.44 mmol) at 0 °C. The reaction mixture was stirred at rt for 6 h, quenched with an aq solution of ascorbic acid (0.7%), citric acid (1.3%) and NaOH (0.9%) (20 mL) according to Ogawa's procedure [7]. The mixture was filtered on Celite and the filtrate was washed with aq $NaHCO_3$ and satd NaCl. Evaporation and filtration of the residue on silica (3:1 petroleum ether–EtOAc) gave the intermediate mixed acetal contaminated with some starting material (550 mg).

This mixture was taken up in CH_2Cl_2 (35 mL) and stirred with 3 Å molecular sieves and IDCP (600 mg, 1.28 mmol) for 20 min at rt. A solution of Me_3SiOTf (1 M in toluene, 107 μL, 0.107 mmol) was added and the reaction left to proceed for 2 h. After neutralization with triethylamine, the salts were filtered on Celite and the filtrate evaporated. Chromatography (1:1 petroleum ether–EtOAc) gave **9** as a slightly yellow oil (200 mg, 51%); $[\alpha]_D^{25}$ −95° (*c* 1.04, $CHCl_3$); ^1H NMR data see Table 2; ^{13}C NMR ($CDCl_3$): δ 170.98, 170.68 (C=O), 102.33 (C-1), 79.37 (C-3), 79.23 (C-4), 76.49 (C-2), 65.38 (C-5), 55.52 (O*C*H_3), 20.85, 20.81 (*C*H_3CO); ISMS: *m*/*z* 271 [M + Na]. Anal. Calcd for $C_{10}H_{16}O_7$: C, 48.38; H, 6.50. Found: C, 48.71; H, 6.46.

Table 2
^{1}H NMR chemical shifts (δ), apparent multiplicities and coupling constants in Hz for β-D-arabinofuranoside derivatives **9**–**13**

H	**9**	**10**	**11**	**12**	**13**
1	4.90 d $J_{1,2}$ 4.5	4.73 d $J_{1,2}$ 4.0	4.80 d $J_{1,2}$ 4.5	4.71 d $J_{1,2}$ 4.2	4.62 d $J_{1,2}$ 4.5
2	4.27 ddd $J_{2,3}$ 6.2 $J_{2,OH}$ 8.5	3.92 m	4.14 m	4.07 dd $J_{2,3}$ 6.5	4.07 dd $J_{2,3}$ 7.5
3	5.05 dd $J_{3,4}$ 5.0	3.92 m	4.05 m	4.19 dd $J_{3,4}$ 5.2	4.26 dd $J_{3,4}$ 6.0
4	4.11 ddd $J_{4,5a}$ 3.2 $J_{4,5b}$ 7.0	3.76 ddd $J_{4,5a}$ 4.0 $J_{4,5b}$ 7.0	3.89 ddd J 4.5, 5.0, 7.5	4.05 m	4.04 m
5a	4.16 dd $J_{5a,5b}$ 11.0	3.54 dd $J_{5a,5b}$ 11.5	3.77 d $J_{5a,4}$ 5.0	3.73 d $J_{5a,4}$ 6.2	3.56 dd $J_{5a,4}$ 5.5 $J_{5a,5b}$ 12.0
5b	4.37 dd	3.66 dd	3.77 d	3.73 d	3.69 dd $J_{5b,4}$ 3.5
Other signals	OCH_3 3.48 s OAc 2.09, 2.12, s OH 2.80 d	OCH_3 3.40 s	OCH_3 3.36 s CH_3 1.05 s	OCH_3 3.26 s CH_3 1.04 s	OCH_3 3.40 s OH 2.15 m

Methyl 2,3-di-O-*benzyl-β*-D-*arabinofuranoside* (**13**).—Deacetylation of **9** (220 mg, 0.88 mmol) was carried out as described above for the preparation of **4**. Chromatography (10:1 CH_2Cl_2–MeOH) gave methyl β-D-arabinofuranoside (**10**) (115 mg, 80%); $[\alpha]_D^{25}$ −114° (*c* 1.05, MeOH), lit. −117° [35]; ^{1}H NMR data see Table 2. Compound **10** (88 mg, 0.54 mmol) in DMF (4 mL) was treated at rt with imidazole (48 mg, 0.70 mmol) and *tert*-butyldiphenylsilyl chloride (150 μL, 0.58 mmol) and the mixture was heated at 60 °C for 1 h. Work-up and chromatography (1:2 petroleum ether–EtOAc) gave methyl 5-*O*-*tert*-butyldiphenylsilyl-β-D-arabinofuranoside (**11**) as a colorless oil (140 mg, 65%); $[\alpha]_D^{25}$ −48° (*c* 1.04, $CHCl_3$); ^{1}H NMR data see Table 2; ^{13}C NMR ($CDCl_3$): δ 133.55, 133.20, 129.81, 129.77, 127.74, 125.84 (Ar), 101.75 (C-1), 81.82 (C-4), 78.04 (C-3), 65.03 (C-5), 55.37 (O*C*H$_3$), 26.80, 19.24 (*t*Bu).

A solution of **11** (87 mg, 0.216 mmol) and benzyl bromide (56 μL, 0.47 mmol) in DMF (3 mL) was treated at 0 °C with NaH (60% dispersion in mineral oil, 32 mg, 0.86 mmol). The reaction was left at rt for 4 h, quenched with MeOH and a few drops of 1 M HCl before concentration. Chromatography (5:1 petroleum ether–EtOAc) gave methyl 2,3-di-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-β-D-arabinofuranoside (**12**) as a colorless oil (110 mg, 87%); $[\alpha]_D^{25}$ −24° (*c* 1.04, $CHCl_3$); ^{1}H NMR data see Table 2; ^{13}C NMR ($CDCl_3$): δ 135.58, 129.68, 128.37, 128.31, 128.18, 127.90, 127.68, 127.58, 126.86 (Ar), 101.51 (C-1), 84.29, 83.32, 82.04 (C-2, C-3, C-4), 72.52, 72.37 (O*C*H$_2$Ph), 66.01 (C-5), 54.93 (O*C*H$_3$), 29.70, 26.82 (*t*Bu).

Compound **12** (150 mg, 0.257 mmol) was treated in THF (3 mL) with *n*Bu$_4$NF (1 M in THF, 290 μL, 0.29 mmol) for 40 min at rt. Work-up and chromatography (2:1 petroleum ether–EtOAc) gave **13** as a slightly yellow oil (65 mg, 74%); $[\alpha]_D^{25}$ −38° (*c* 1.03, $CHCl_3$); ^{1}H NMR data see Table 2; ^{13}C NMR ($CDCl_3$): δ 137.91, 137.47, 128.42, 128.18, 128.01, 127.81, 127.76, 125.84 (Ar), 101.83 (C-1), 84.31 (C-2), 82.22 (C-4), 80.97 (C-3), 72.67, 72.58 (O*C*H$_2$Ph), 63.99 (C-5), 55.70 (O*C*H$_3$). Anal. Calcd for $C_{20}H_{24}O_5$: C, 69.75; H, 7.02. Found: C, 69.56; H, 7.21.

Methyl 2,3-di-O-*acetyl-5-(3,5-di*-O-*acetyl-β*-D-*arabinofuranosyl)-α*-D-*arabinofuranoside* (**14**).—A mixture of **8** (50 mg, 0.125 mmol) and methyl 2,3-di-*O*-acetyl-α-D-arabinofuranoside (32.5 mg, 0.132 mmol) was stirred with activated 4 Å molecular sieves in CH_2Cl_2 for

20 min before addition of DDQ (42.5 mg, 0.187 mmol) at 0 °C. The reaction was left 3 h at rt and treated as described above for the preparation of **9**. Chromatography (4:1 then 1:1 petroleum ether–EtOAc) gave the intermediate acetal as a colorless oil (57 mg, 71%). This oil was treated as above in CH_2Cl_2 (1.6 mL) containing IDCP (48 mg, 0.102 mmol) and 4 Å molecular sieves. Glycosylation, promoted with Me_3SiOTf (8.5 μL, 0.008 mmol) was over in 45 min at rt. Work-up and chromatography (1:2 petroleum ether–EtOAc) gave **14** as a yellow oil (29 mg, 52% from **8**); $[\alpha]_D^{25}$ −22° (*c* 1.40, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.12 (d, 1 H, $J_{1',2'}$ 4.5 Hz, H-1′), 5.10 (d, 1 H, $J_{2,3}$ 1.2 Hz, H-2), 5.09 (dd, 1 H, $J_{2',3'}$ 6.5, $J_{3',4'}$ 5.0 Hz, H-3′), 5.03 (dd, 1 H, $J_{3,4}$ 4.5 Hz, H-3), 4.93 (s, 1 H, H-1), 4.37 (dd, 1 H, $J_{5a',5b'}$ 11.2, $J_{5a',4'}$ 4.0 Hz, H-5a′), 4.31 (dd, 1 H, H-2′), 4.20 (dd, 1 H, $J_{5b',4'}$ 7.5 Hz, H-5b′), 4.11 (dd, 1 H, $J_{5a,5b}$ 10.8, $J_{5a,4}$ 3.0 Hz, H-5a), 4.20–4.10 (m, 2 H, H-4 and H-4′), 3.76 (dd, 1 H, $J_{5b,4}$ 4.2 Hz, H-5b), 3.40 (s, 3 H, OMe), 2.09–2.12 (4s, 12 H, OAc); ^{13}C NMR ($CDCl_3$): δ 170.66, 170.47, 169.79 (C=O), 106.71 (C-1), 101.54 (C-1′), 82.01, 81.35, 79.41, 78.97, 77.19, 67.19, 65.48 (C-5, C-5′), 54.93 (O*C*H$_3$). Anal. Calcd for $C_{19}H_{28}O_{13}$: C, 49.14; H, 6.08. Found: C, 48.78; H, 5.92.

*1*L-*1,2,4,5,6-Penta*-O-*benzyl*-myo-*inositol 3-hydrogen phosphonate* (**16**).—A solution of imidazole (220 mg, 3.23 mmol) in anhyd toluene (2.7 mL) was treated at 0 °C with PCl_3 (62 μL, 0.71 mmol in 0.65 mL of toluene) and Et_3N (220 μL, 1.58 mmol in 0.65 mL of toluene). After 10 min, the temperature was lowered to −5 °C and a solution of **15** (150 mg, 0.24 mmol) in 4.5 mL of toluene was added dropwise over 1 h. The reaction mixture was left for 1 h and allowed to warm to 15 °C before quenching with aq pyridine (4:1 pyridine–water, 35 mL); CH_2Cl_2 extraction and chromatography (8:1 CH_2Cl_2–MeOH) gave **16** as a white foam (97 mg, 57%); $[\alpha]_D^{25}$ +22° (*c* 1.49, $CHCl_3$); ^{1}H NMR (9:1 $CDCl_3$–CD_3OD): δ 7.76–6.90 (m, 25 H, Ar), 6.91 (d, 1 H, $J_{P,H}$ 642 Hz, H–P), 7.94–4.72 (m, 8 H, CH_2Ph), 4.63, 4.58 (2d, 2 H, *J* 11.0 Hz, CH_2Ph), 4.23 (br t, 1 H, $J_{1,2}$ 2.2, $J_{2,3}$ 2.2 Hz, H-2), 4.19–3.95 (m, 3 H, H-4, H-5, H-6), 3.44 (m, 2 H, H-1, H-3); ^{13}C NMR (9:1 $CDCl_3$–CD_3OD): δ 138.61, 138.32, 138.23, 138.01, 137.95, 128.17, 127.94, 127.52, 127.43, 127.23, 121.47, 83.02, 81.25, 80.64, 80.47, 75.65, 75.50, 74.68, 72.18; ^{31}P NMR (9:1 $CDCl_3$–CD_3OD): δ 8.80; ESMS: *m*/*z* 693 [M − 1]. Anal. Calcd for $C_{41}H_{43}O_8P$: C, 70.88; H, 6.24. Found: C, 70.72; H, 6.08.

*1*D-*1,2,4,5,6-Penta*-O-*benzyl*-myo-*inositol 3-hydrogen phosphonate* (**18**).—Starting from **17** (150 mg, 0.24 mmol) and following the procedure described above for the preparation of **16**, compound **18** was obtained as a white foam (95 mg, 56%); $[\alpha]_D^{25}$ −23° (*c* 1.65, $CHCl_3$); for NMR data, see **16**; ESMS: *m*/*z* 693 [M − 1]. Anal. Calcd for $C_{41}H_{43}O_8P$: C, 70.88; H, 6.24. Found: C, 70.82; H, 6.07.

Methyl 2,3-di-O-*benzyl-5-(1*L-*1,2,4,5,6-penta*-O-*benzyl*-myo-*inositol 3-phosphate)* β-D-*arabinofuranoside* (**19**).—A solution of **16** (20 mg, 0.028 mmol) and **13** (8.8 mg, 0.025 mmol) in anhyd pyridine (0.75 mL) was stirred for 20 min with activated 4 Å molecular sieves before addition of pivaloyl chloride (9.5 μL, 0.077 mmol). After 20 h at rt, a solution of iodine (13 mg, 0.051 mmol) in 98% aq pyridine (0.3 mL) was added and the mixture left for an additional 1 h before filtration on Celite. The filtrate was diluted with CH_2Cl_2 and washed with satd sodium thiosulfate. Chromatography (15:1 CH_2Cl_2–MeOH) gave **19** as a colorless oil (17.5 mg, 67%); $[\alpha]_D^{25}$ −4.8° (*c* 0.87, $CHCl_3$); ^{1}H NMR (9:1 $CDCl_3$–CD_3OD): δ 7.35–7.03 (m, 35 H, Ar), 4.87–4.41 (m, 15 H, CH_2Ph, H-1), 4.37 (s, 1 H, H-2′), 4.05–3.84 (m, 8 H, H-1′, H-4′, H-6′, H-2, H-3, H-4, H-5), 3.35 (dd, 1 H, $J_{3',4'}$ 9.5, $J_{3',2'}$ 2.0 Hz, H-3′), 3.28 (t, 1 H, $J_{5',6'} \sim J_{5',4'}$ 9.5 Hz, H-5′), 3.17 (s, 3 H, OCH_3); ^{13}C NMR (9:1 $CDCl_3$–CD_3OD): δ 138.32, 137.67, 137.24, 128.48, 128.16, 127.94, 127.55, 127.40 (Ar), 101.69 (C-1), 83.82, 82.98, 82.70, 81.40, 80.69, 80.60, 80.33, 75.72, 74.81, 72.37, 72.20, 72.03, 67.97, 54.78; ^{31}P NMR (9:1 $CDCl_3$–CD_3OD, 303 K): δ 0.67; ESMS: *m*/*z* 1036 [M − 1].

Methyl 2,3-di-O-*benzyl-5-(1*D-*1,2,4,5,6-penta*-O-*benzyl*-myo-*inositol 3-phosphate)*-β-D-*arabinofuranoside* (**20**).—Starting from **18** (20 mg, 0.028 mmol) and **13** (8.8 mg, 0.025 mmol) and following the procedure described above for the preparation of **19**, compound **20** was obtained (18 mg, 69%); $[\alpha]_D^{25}$ −26°

Table 3
^{1}H NMR chemical shifts (δ), apparent multiplicities, coupling constants in Hz, and ^{13}C NMR chemical shifts (δ) for compounds **1** and **2** (D_2O, 303 K)[a]

	H-1	H-2	H-3	H-4	H-5	H-5
1	4.90, d $J_{1,2}$ 4.2	4.14, dd $J_{2,3}$ 7.5	4.09, dd $J_{3,4}$ 6.0	4.01, m	4.07, m	3.95, m
2	4.89, d $J_{1,2}$ 4.5	4.14, dd $J_{2,3}$ 8.0	4.09, m $J_{3,4}$ 6.0	4.00, m	4.04, m	3.97, m
	H-1′	H-2′	H-3′	H-4′	H-5′	H-6′
1	3.94, ddd $J_{1',2'}$ 2.7 $J_{1',P}$ 11	4.25, t $J_{2',3'}$ 2.7	3.54, dd $J_{3',4'}$ 9.5	3.64, t $J_{4',5'}$ 9.5	3.31, t $J_{5',6'}$ 9.5	3.74, t $J_{6',1'}$ 9.5
2	3.95, ddd $J_{1',2'}$ 2.8	4.25, t $J_{2',3'}$ 2.8	3.53, dd $J_{3',4'}$ 9.5	3.63, t $J_{4',5'}$ 9.5	3.31, t $J_{5',6'}$ 9.5	3.74, t $J_{6',1'}$ 9.5
LAM	3.98, t $J_{1',2'}$ 2.2 $J_{1',P}$ 9.6	4.28, s $J_{2',3'}$ 2.2	3.58, d $J_{3',4'}$ 9.2	3.67, t $J_{4',5'}$ 9.2	3.35, t $J_{5',6'}$ 9.6	3.77, t $J_{6',1'}$ 9.6
	C-1	C-2	C-3	C-4	C-5	
1	103.45	77.15	75.38	81.52	67.50	
2	103.18	77.18	75.42	81.51	67.46	
	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
1	77.29	72.19	71.62	73.21	74.89	72.35
2	77.32	72.28	71.82	73.28	74.99	72.47
LAM	77.37	72.20	71.61	73.13	74.89	72.24

[a] Values for the LAM are taken from Ref. [5].

(c 1.11, $CHCl_3$); ^{1}H NMR (9:1 $CDCl_3$–CD_3OD): δ 7.35–7.04 (m, 35 H, Ar), 4.88–4.42 (m, 15 H, CH_2Ph, H-1), 4.37 (s, 1 H, H-2′), 4.04–3.90 (m, 8 H, H-1′, H-4′, H-6′, H-2, H-3, H-4, H-5), 3.36 (dd, 1 H, $J_{3',4'}$ 9.5, $J_{3',2'}$ 2.0 Hz, H-3′), 3.29 (t, 1 H, $J_{5',6'} \sim J_{5',4'}$ 9.5 Hz, H-5′), 3.18 (s, 3 H, OCH_3); ^{13}C NMR (9:1 $CDCl_3$-CD_3OD): δ 138.92, 138.41, 138.26, 138.14, 137.82, 137.59, 137.18, 128.27, 128.13, 128.01, 127.93, 127.85, 127.53, 127.35, 127.29, 127.11 (Ar), 101.54 (C-1), 83.78, 82.91 (C-5′), 82.26, 81.35, 80.88, 80.46, 80.34 (C-3′), 76.37 (C-2′), 76.11, 75.81, 75.70, 74.77, 72.31, 72.10, 71.98, 67.72 (C-5), 54.70 (OCH_3); ^{31}P NMR (9:1 $CDCl_3$–CD_3OD, 303 K): δ 0.67; ESMS: m/z 1036 [M − 1].

*Methyl β-*D*-arabinofuranoside 5-(1*D*-myo-inositol 1-phosphate)* (**1**).—Compound **19** (30 mg, 0.029 mmol) in 1:2:1 EtOAc–MeOH–H_2O (0.5 mL) was hydrogenolyzed on Pd-(OH)$_2$–C for 8 h. The catalyst was filtered on Celite and the residue was chromatographed on silica (1:2:1 EtOAc–MeOH–H_2O) to give **1** as a white foam (6.5 mg, 56%); for ^{1}H NMR and ^{13}C NMR data, see Table 3; ^{31}P NMR (D_2O, 303 K): δ 5.52; ESMS: m/z 405 [M − 1].

*Methyl β-*D*-arabinofuranoside 5-(1*L*-myo-inositol 1-phosphate)* (**2**).—Hydrogenolysis of **20** (30 mg, 0.029 mmol), as above, gave **2** as a white foam (10.5 mg, 79%); for ^{1}H NMR and ^{13}C NMR data, see Table 3; ^{31}P NMR (D_2O, 303 K): δ 5.52; ESMS: m/z 405 [M − 1].

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