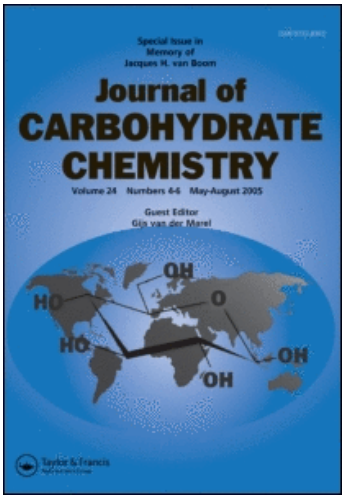

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Synthesis and Serological Properties of Methyl 2,6-Dideoxy-4-O-Me-α-D and L- arabino - hexopyranoside Present in the Glycolipid Phenolic Antigen of Mycobactkrium Kansasii

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SYNTHESIS AND SEROLOGICAL PROPERTIES OF METHYL 2,6-DIDEOXY-4-O-Me-α-D and L- arabino - HEXOPYRANOSIDE PRESENT IN THE GLYCOLIPID PHENOLIC ANTIGEN OF MYCOBACTERIUM KANSASII

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ABSTRACT

Methyl 2,6-dideoxy-4-O-methyl-α-D- arabino -hexopyranoside 13 and its L-enantiomer 14 were synthesized in a 5-step sequence starting from either 6-deoxy-D-glucal or L-rhamnal. The D-enantiomer was proven to be identical with the non-reducing sugar of the tetrasaccharide moiety of the major phenol glycolipid, a cell wall antigen of Mycobacterium kansasii.

INTRODUCTION

Mycobacteria are important pathogens of man[1] and animals.[2] The notorious ones are M. tuberculosis[3] and M. leprae[4] which are respectively the etiologic agents of over 20 million cases of human tuberculosis and leprosy mainly located in the developing world. Other atypical mycobacterial, mainly the M. avium complex and M kansasii are also the causes of human diseases resembling tuberculosis.[5] These opportunistic pathogens have also been found to be the etiological agents of disseminated infections in patients with acquired immunodeficiency syndrome[6] (AIDS).

At the present time, the identification of the mycobacteria responsible for patient infections is based on bacteriological culture tests. The search for serological tests is an important challenge aiming for an earlier diagnosis of mycobacterial infections. Such a strategy permits more specific chemotherapy at the start of an infection. Mainly from Goren and Brennan's research[7,8] three classes of glycolipids : the C-mycoside glycopeptidolipids, the trehalose containing lipooligosaccharides (LOS) and the phenolic glycolipids (Phe Gl), are the specific immunoreactive compounds of non-tuberculous mycobacterial pathogens.

The M. kansasii walls contain both phenolic glycolipids[9] and LOS[10] antigens. The major Phe Gl oligosaccharide moiety is a tetrasaccharide containing the 2,6-dideoxy-4-O-methyl-α-D-arabino--hexopyranosyl residue.[11,12]

The present manuscript reports the synthesis of the methyl 2,6-dideoxy-4-O-methyl-α-D-arabino-hexopyranoside as well as its L-enantiomer. By serological tests using enzyme-linked immunosorbent assays (ELISA) only the D-enantiomer was shown to partially inhibit the immunochemical linkage of the phenolic glycolipid towards rabbit polyclonal antibodies anti-Phe Gl.

RESULTS AND DISCUSSION

Synthesis

Methyl 2,6-dideoxy-4-O-methyl- α -D-arabino-hexopyranoside 13 and its L-enantiomer 14 were synthesized according to the sequence depicted in Scheme I.

1,5-Anhydro-2,6-dideoxy-D-arabino-hex-1-enitol 1 (6-deoxy-D-glucal) was prepared from the commercially available 3,4,6-tri-O-acetyl-D-glucal.[13] Methoxymercuration of compound 1 was performed as previously reported[14] for the enantiomeric L-rhamnal[15] and yielded the crystalline methyl 2-acetoxymercurio-2,6-dideoxy- α -D-mannopyranoside 3 (48 % after recrystallization from MeOH). Reductive demercuration of 3 with methanolic sodium borohydride gave methyl 2,6-dideoxy- α -D-arabino-hexopyranoside 5. The high values (9.0-9.5 Hz) of $J_{3,4}$ and $J_{4,5}$ in the ^1H NMR spectrum of 3 and its 3,4-diacetate 6 indicate a $^4C_1$-D conformation for each compound and the low value (3.5 Hz) of $J_{1,2}$ indicates MeO-1 and AcOHg-2 to be trans-diaxial (Table I). Methoxymercuration followed by reductive demercuration[14,15] of 1,5-anhydro-2,6-dideoxy-L-arabino-hex-1-enitol 2 (L-Rhamnal) yielded methyl 2,6-dideoxy- α -L-arabino-hexopyranoside 7 (61 % after recrystallization from MeOH).

Regioselective p-toluenesulfonylation of either compound 5[16] or its enantiomer[17,23] 7 has been already reported. Similarly, treatment of the glycosides 5 and 7 with tert-butyldiphenylchlorosilane in N,N-dimethylformamide in the presence of imidazole[18] gave the corresponding 3-O-silylated derivatives 8 and 10 in high yield. When the silylether 8 was acetylated, the signal for H-4 in the NMR spectrum of compound 9 was shifted to lower field by 1.57 ppm (Table I) indicating that silylation had occured exclusively at the O-3 position.

Surprisingly, the free hydroxyl group in both compounds 8 and 10 exhibited poor reactivity. Methylation using sodium hydride as a base in tetrahydrofuran yielded a mixture of products, due to

the migration of the silyl protecting group and exhaustive desilylation. When n-BuLi (1.0 eq.) was used in order to generate the alcoholate, methylation occured but the alkylation process stopped after 20 to 40 % conversion of the starting alcohol. The high reactivity of methyl trifluoromethanesulfonate (methyl triflate) make it a suitable reagent for methylation of carbohydrates under mild conditions.[19,20] Indeed, treatment of compounds 8 and 10 with methyl triflate in the presence of the strongly sterically hindered base 2,6-di-tert-butylpyridine afforded the methylated derivatives 11 and 12 in 86 % yield. According to this process, a large excess of the alkylating reagent and of the expensive base were needed. Use of n-BuLi as a base provided an econimic alternative for this alkylation step. Although the yields were lowered to 60-70 %, the unreacted alcohols could be recovered then reprocessed.

Finally, compounds 11 and 12 were desilylated using n-tetrabutylammonium fluoride in tetrahydrofuran.[21] Compound 11 afforded the new methyl 2,6-dideoxy-4-O-methyl-α-D-arabino-hexopyranoside 13 as a crystalline material. Its physical data were in full agreement with the expected structure. Similarly, compound 12 afforded the known L-enantiomer 14.[22,23] This latter product was previously reported as a crystalline material first by Yoshimura et al. [22] then by Monneret et al.[23] Our short synthesis of both D- and L-enantiomers 13 and 14 starting either from 6-deoxy-D-glucal 1 or from L-rhamnal 7 using the regioselective silylation described above, offers an alternative route to the previously reported syntheses for compound 14.[24,25]

Serological properties

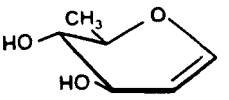
The 2,6-dideoxy-4-O-methyl-arabino-hexopyranosyl residue has been found as the non-reducing terminal carbohydrate of Phe Gl K-I[11], the major antigen of M. kansasii. We used this biological property to prepare rabbits polyclonal anti-Phe Gl K-I sera, whose specificity in the mycobacterial genus was shown to be restricted to the kansasii and gastri species.[26]

TABLE I

^{1}H NMR (300 MHz) data

Compound	Chemical shifts (δ) reported in ppm downfield from TMS as an internal standard									
	H-1	H-2e	H-2a	H-3	H-4	H-5	Me-1	Me-4	Me-5	Others
5	4.73	2.13	1.68	3.89	3.11	3.62	3.32	-	1.30	OH 2.3-2.2
6	4.75	2.22	1.76	5.24	4.73	3.82	3.32	-	1.18	Ac 2.04 and 1.99
8 and 10	4.58	1.90	1.69	4.04	3.22	3.51	3.18	-	1.25	Ph 7.73-7.35,t-Bu 1.07, OH 2.04
10 (β)	4.13	1.93	1.64	3.62	3.23	3.15	3.38	-	1.31	Ph 7.73-7.35,t-Bu 1.05, OH 2.10
9	4.57	1.87	1.71	4.21	4.79	3.60	3.16	-	1.12	t-Bu 1.06, Ac 1.85
11 and 12	4.47	1.65	1.53	4.18	2.81	3.50	3.12	3.54	1.27	Ph 7.76-7.32,t-Bu 1.07
11 (β)	4.02	1.71	1.52	3.79	2.83	3.15	3.33	3.58	1.33	Ph 7.77-7.30,t-Bu 1.07
13 and 14	4.71	2.12	1.68	3.94	2.70	3.60	3.30	3.57	1.30	OH 1.54 (2.34)

Compound	First-order coupling constants (Hz)							
	$J_{1,2e}$	$J_{1,2a}$	$J_{2e,3}$	$J_{2a,3}$	$J_{2e,2a}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$
5	1.0	3.5	5.0	11.5	12.5	9.0	9.0	6.5
6	1.0	3.5	5.5	11.5	12.5	9.5	9.5	6.5
8 and 10	1.0	3.2	5.0	11.5	12.5	9.0	9.0	6.2
10 (β)	2.0	10.0	5.0	11.5	12.5	9.0	9.0	6.5
9	1.0	3.5	5.0	11.0	12.5	9.4	9.4	6.3
11 and 12	1.5	3.5	5.5	11.0	12.5	9.0	9.0	6.3
11 (β)	2.0	10.0	5.0	11.5	12.5	9.0	9.0	6.0
13 and 14	1.0	3.5	5.0	11.5	12.5	9.2	9.2	6.3



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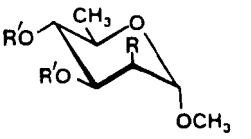
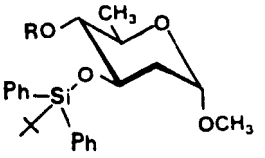


3 R = HgOAc, R′= H

5 R = H, R′= H

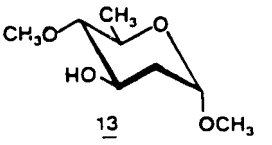
6 R = H, R′= Ac



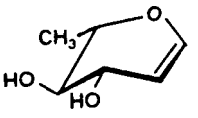
8 R = H

9 R = Ac

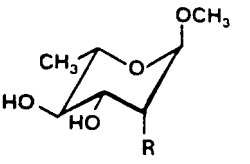
11 R = CH_3



13

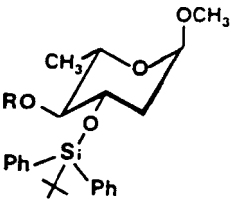


2



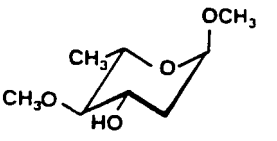
4 R = HgOAc

7 R = H



10 R = H

12 R = CH_3



14

Scheme 1

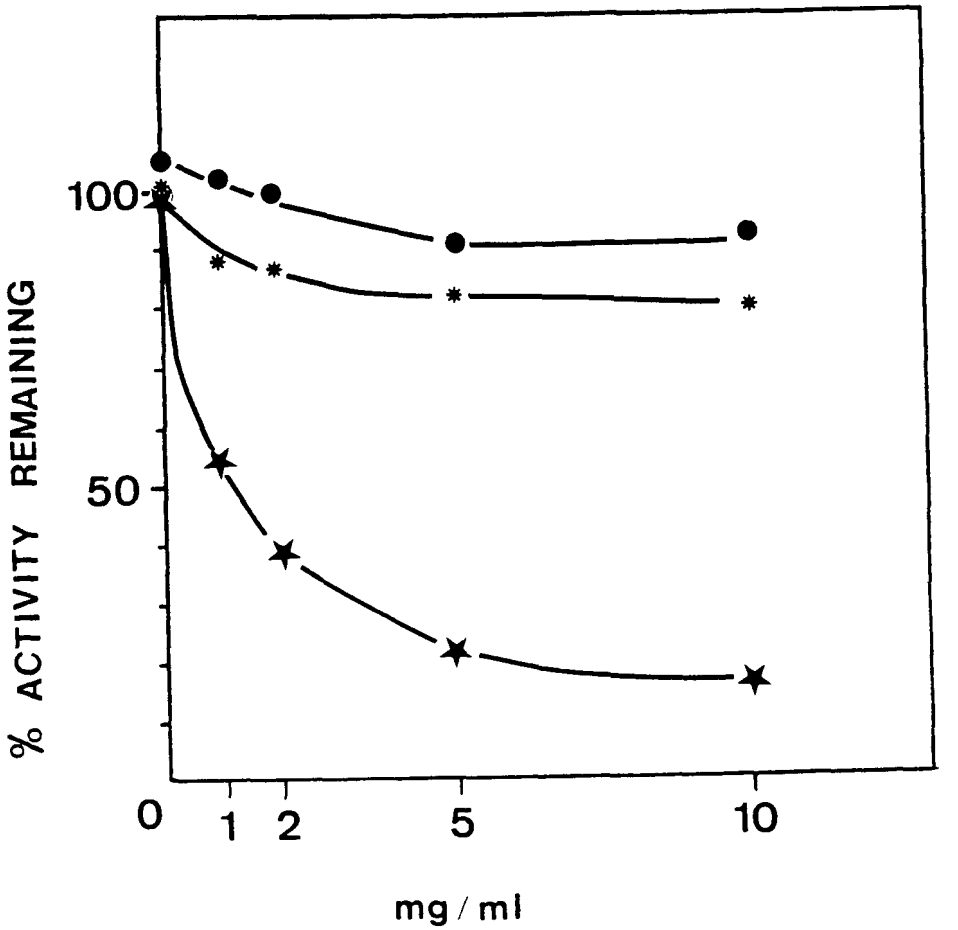


Fig. 1 : ELISA inhibition activity of the synthetic monosaccharides against rabbit polyclonal anti-Phe Gl K-I sera. The general protocol is described in the experimental section.

★ monosaccharide 13, ✸ monosaccharide 14, ● methyl α-D-glucopyranoside, ● methyl α-D-galactopyranoside.

The antigenic properties of compounds 13 and 14 towards this anti-Phe Gl K-I serum were analyzed according to two ELISA methods. In a first procedure, these monosaccharides were coated in increasing amounts[11] (from 3 μg/mL to 1 mg/mL, 50 μL per well) and compared to the native Phe Gl K-I antigen (3 μg/mL, 50 μL per well). No activity could be detected at any monosaccharide concentration (average DO 405 nm = 0.03 ± 0.02) compared to the Phe Gl K-I positive reaction (0.720 ± 0.02). From these data, compounds 13 and 14 appeared inactive using direct ELISA protocol.

The second procedure used was the inhibition ELISA method.[27,28] After Phe Gl K-I coating, following the usual technique, the anti-Phe Gl K-I serum was added together in solution with compounds 13 or 14. The monosaccharide concentrations in PBS buffer containing 1 % of BSA were : 0, 10^{-3}, 10^{-2}, 1, 2, 5 and 10 mg/mL. After overnight incubation the adsorbed immune complexes were quantified following the classical β-galactosidase conjugate system. By this technique the inhibiting activities of methyl α-D-glucopyranoside, methyl α-D-galactopyranoside and monosaccharides 13 and 14 were compared (Fig. 1).

Figure 1 shows no inhibiting activity of methyl α-D-glucopyranoside, methyl α-D-galactopyranoside and monosaccharide 14 at any concentration tested while at a 10 mg/mL concentration the monosaccharide 13 inhibits almost totally the Phe Gl K-I specific binding. 50 % of the activity remaining is obtained with about 1 mg/mL i.e. at 6.2 mM. So, the 2,6-dideoxy-4-O-methyl-α-D-arabino-hexopyranosyl residue behaves as the immunodominant epitope of the Phe Gl K-I antigen.

EXPERIMENTAL

Synthesis

General methods : Melting points were determined on an Electrothermal digital melting point apparatus and are corrected. ^{1}H NMR spectra were recorded on a Bruker AM 300 WB instrument. Mass spec-

tra were determined on a Ribermag R-10- 10 C instrument using NH_3 desorption chemical ionization mode. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter at ambient temperature. IR spectra were recorded on a Perkin-Elmer Model 177 spectrophotometer and are reported in wave numbers (cm^{-1}).

Analytical TLC was performed on 0.25 mm pre-coated silica gel plates purchased from E. Merck. Products were purified using the flash chromatography technique on Kieselgel 60 (230-400 mesh ASTM, 0.040-0.063 mm) purchased from E. Merck.

Commercial grade reagents and solvents were used as supplied with the following exceptions : methylene chloride, distilled over phosphorus pentoxide ; ether and hexanes, distilled over calcium hydride ; N,N-dimethylformamide (DMF), dried over Linde type 4A molecular sieves then distilled under reduced pressure and tetrahydrofuran (THF), distilled over sodium benzophenone ketyl.

Every reaction sensitive to oxygen or moisture was run under an argon atmosphere.

Elemental analyses were obtained from the Service Central d'Analyse du Centre National de la Recherche Scientifique (Vernaison, France).

Methyl 2-acetoxymercurio-2,6-dideoxy-α-D-mannopyranoside 3

Quantitative methoxymercuration of 6-deoxy-D-glucal[13] 1 (3.00 g, 23.1 mM) was achieved according to Ref. 14. Pure compound 3 was obtained after two recrystallizations from methanol (4.66 g, 48 %), mp 146.4°C ; $[\alpha]_D^{23}$ = + 5° (c 0.63, Methanol).

Anal. Calcd for $C_9 H_{16} Hg O_6$: C, 25.69 ; H, 3.83. Found : C, 25.99 ; H, 4.00.

Methyl 2-acetoxymercurio-2,6-dideoxy-α-L-mannopyranoside 4

Pure L-enantiomer 4 was obtained from quantitative methoxymercuration of L-Rhamnal[15] 2 (1.21 g, 9.62 mM) according to Ref. 14 (2.48 g, 61 % after two recrystallizations from methanol), mp 145°C, $[\alpha]_D^{23}$ = - 5° (c 1.28, Methanol) ; (lit.[14] mp 150-152°C (from acetone), $[\alpha]_D^{23}$ = - 5° (c 0.57, Methanol), lit.[15] mp 145-150°C (from methanol-ether), $[\alpha]_D^{23}$ = - 2° (c 1.00, Methanol).

Methyl 2,6-dideoxy-α-D-arabino-hexopyranoside 5[16,29,30]

Reductive demercuration of compound 3 (3.01 g, 7.15 mM) was performed according to Ref. 14 to yield the syrupy dideoxy glycoside 5 (1.05 g, 90.7 %), $[\alpha]_D^{23}$ = + 149° (c 0.54, Acetone) ; (lit.[29] $[\alpha]_D^{23}$ = + 120° (c 2.0, Water) ; ^{1}H NMR (Table I) ; MS (C_7 H_{14} O_4, M.W. = 162) : 180 (26) $(M + NH_4)^+$, 148 (100) $(M + NH_4 - CH_3OH)^+$.

A sample of compound 5 was acetylated (acetic anhydride, pyridine) to give the diacetate 6 ; $[\alpha]_D^{23}$ = + 138° (c 0.49, Acetone); ^{1}H NMR (Table I) ; MS (C_{11} H_{18} O_6, M.W. = 246) : 264 (100) $(M + NH_4)^+$.

Anal. Calcd for C_{11} H_{18} O_6 : C, 53.65 ; H, 7.37. Found : C, 53.55 ; H, 7.26.

Reductive demercuration of the mother liquors of compound 3 gave a syrupy product, $[\alpha]_D^{23}$ = + 52° (c 0.71, Acetone) indicating this product to be a mixture of the two anomers α- and β- 5.

Methyl 2,6-dideoxy-α-L-arabino-hexopyranoside 7

Pure L-enantiomer 7 was obtained from reductive demercuration[14] of the crystalline compound 4 (2,48 g, 5.90 mM) as a pale yellow syrup (768 mg, 81 %), $[\alpha]_D^{23}$ = - 128° (c 1.09, Acetone) ; (lit.[14] $[\alpha]_D^{23}$=-146° (c 0.73, Acetone), lit.[15] $[\alpha]_D^{23}$=-113° (c 0.7, Water).

Methyl 3-O-tert-butyldiphenylsilyl-2,6-dideoxy-α-D-arabino-hexopyranoside 8

tert-Butylchlorodiphenylsilane (2.9 mL, 11.2 mM, 1.05 eq) was added into a solution of diol 5 (1.73 g, 10.7 mM) in dry DMF (3.4 mL) containing imidazole (1.81 g, 26.6 mM, 2.5 eq)[18]. The homogeneous solution was warmed to 40°C overnight. The reaction mixture was concentrated and the residue was suspended in ether (200 mL). The organic phase was successively washed with brine containing 1 N aqueous hydrochloric acid, brine containing 10 % aqueous sodium hydrogen carbonate and then dried over $MgSO_4$. Concentration of the solution afforded crude silyl ether 8. Purification using flash chromatography (SiO_2 : 50 g, elution with hexanes-ethyl acetate 9:1 containing 0.1 % Et_3N) afforded pure 8 as a thick

syrup (3.80 g, 89 %), $[\alpha]_D^{23}$ = + 58° (c 1.47, Acetone) ; ^1H NMR (Table I) ; IR (neat) : 3580 and 3540-3400 (broad, OH), 3070, 3050, 1590 and 700 (Ph) ; MS (C_{23} H_{32} O_4 Si, M.W. = 400) : 418 (9) $(M + NH_4)^+$, 386 (32) $(M + NH_4 - CH_3OH)^+$, 369 (9) $(MH - CH_3OH)^+$.

Anal. Calcd for C_{23} H_{32} O_4 Si : C, 68.96 ; H, 8.05. Found : C, 68.73 ; H, 7.88.

A sample of compound 8 was acetylated (acetic anhydride, pyridine) to give the monoacetate 9, $[\alpha]_D^{23}$ = + 74° (c, 0.33, Acetone); ^1H NMR (Table I) ; MS (C_{25} H_{34} O_5 Si, M.W. = 442) : 460 (100) $(M + NH_4)^+$.

Anal. Calcd for C_{25} H_{34} O_5 Si : C, 67.84 ; H, 7.74. Found : C, 67.85 ; H, 7.81.

When the silylation was performed with the anomeric mixture of α and β-5, the two silylated derivatives α - and β - 8 (89 %) could not be separated $[\alpha]_D^{23}$ = + 38° (c 0.73, Acetone).

Methyl 3-O-tert-butyldiphenylsilyl-2,6-dideoxy- α-L-arabino-hexopyranoside 10

Pure L-enantiomer 10 was obtained in a similar way[18] from 7 (768.8 mg, 4.74 mM) as a colourless syrup (1.73 g, 91 %), $[\alpha]_D^{23}$ = - 62° (c 1.07, Acetone) ; ^1H NMR (Table I) ; IR (neat) : 3580 and 3550-3350 (broad, OH), 3070, 3050, 1590 and 700 (Ph) ; MS (C_{23} H_{32} O_4 Si, M.W. = 400) : 418 (33) $(M + NH_4)^+$, 386 (100) $(M + NH_4 - CH_3OH)^+$.

Anal. Calcd for C_{23} H_{32} O_4 Si : C, 68.96 ; H, 8.05. Found : C, 68.87 ; H, 8.01.

When the silylation was carried out with the anomeric mixture of α - and β-7, the two silylated derivatives α-and β-10 could be separated using flash chromatography.

β-10 anomer : $[\alpha]_D^{23}$ = + 20.5° (c 1.10, Acetone), ^1H NMR (Table I).

Methylation of compounds 8 and 10

n-Butyl lithium solution in hexanes was purchased from E. Merck and titrated just prior to being used with 2,5-dimethoxybenzyl alcohol.[31]

Method A : A solution of the alcohol 8 or 10 (1.0 eq) in dry THF (10 mL/g of 8) was cooled to - 30°C. To the homogeneous so-

lution was added 1.6 M n-butyllithium solution in hexanes (1.0 eq) dropwise with a syringe. This solution was stirred at - 30°C for 15 min and then methyl iodide (10.0 eq) was added. The reaction mixture was allowed to warm up and stirring was maintained for 1 h at room temperature. After concentration of the solution under vacuum, the crude reaction mixture was purified by flash chromatography. The 4-O-methylated derivatives 11 or 12 were obtained in 14-40 % yield.

Method B[19] : To a solution of the alcohol 8 or 10 (1.0 eq) in dry methylene chloride (30 mL/g of 8) was first added 2,6-di-tert-butylpyridine (20.0 eq) then methyl trifluoromethanesulfonate (10.0 eq). The homogeneous solution was stirred at 80°C for 36 h. Usual work-up[20] afforded a crude product which was purified by flash chromatography.

Method C : A solution of the alcohol 8 or 10 (1.0 eq) in dry THF (10 mL/g of 8) was cooled to - 30°C. To this homogeneous solution was added 1.6 M n-butyllithium solution in hexanes dropwise with a syringe. After stirring for 15 min, methyl trifluoromethanesulfonate (1.0 eq) was added via a syringe. Stirring was maintained for 5 h at - 30°C after which time the solvents were removed under vacuum. The residue was suspended in ether and the organic phase was washed successively with saturated aqueous sodium hydrogen carbonate, water, brine then dried over $MgSO_4$ and concentrated. The crude material was purified by flash chromatography.

Methyl 3-O-tert-butyldiphenylsilyl-2,6-dideoxy-4-O-methyl-α-D-arabino-hexopyranoside 11

Method A : Reaction of compound 8 (533.0 mg, 1.33 mM) led to compound 11 (120.2 mg, 21.8 %). Further elution with hexanes-ethyl acetate 9:1 yielded the recovered starting material (395.4 mg, 74.2 %).

Method B[19,20] : Reaction of compound 8 (395.4 mg, 0.99 mM) led to compound 11 as a colourless syrup (354.8 mg, 86 %), $[\alpha]_D^{23}$ = + 75° (c 0.74, Acetone) ; ^1H NMR (Table I) ; IR (neat) : 3060, 3040, 1580 and 700 (Ph) ; MS ($C_{24}H_{34}O_4Si$, M.W. = 414) : 432 (39) $(M + NH_4)^+$, 400 (100) $(M + NH_4 - CH_3OH)^+$.

Anal. Calcd for C_{24} H_{34} O_4 Si : C, 69.52 ; H, 8.26. Found : C, 69.53 ; H, 8.38.

When this methylation procedure was applied to the anomeric mixture of α- and β- 8, the two methylated derivatives α- and β -11 could be separated using flash chromatography.

β-11 anomer : $[\alpha]_D^{23}$ = - 8° (c 0.48, Acetone) ; ^{1}H NMR (Table I) ; MS (C_{24} H_{34} O_4 Si, M.W. = 414) : 432 (100) $(M + NH_4)^+$, 400 (74) $(M + NH_4 - CH_3OH)^+$.

Anal. Calcd for C_{24} H_{34} O_4 Si : C, 69.52 ; H, 8.26. Found : C, 69.28 ; H, 8.43.

Method C : Reaction of compound 8 (394.0 mg, 0.98 mM) led to compound 11 (244.7 mg, 60 %) together with recovered starting material (49.3 mg, 12.5 %).

Methyl 3-O-tert-butyldiphenylsilyl-2,6-dideoxy-4-O-methyl- α -L- -arabino-hexopyranoside 12

Reaction of compound 10 (800 mg, 2 mM) according to procedure C led to compound 12 as a colourless syrup (566,5 mg, 68.5 %), $[\alpha]_D^{23}$ = - 78° (c 0.78, Acetone) ; ^{1}H NMR (Table I).

Anal. Calcd for C_{24} H_{34} O_4 Si : C, 69.52 ; H, 8.26. Found : C, 69.27 ; H, 8.23.

Methyl 2,6-dideoxy-4-O-methyl- α-D -arabino-hexopyranoside 13

To a solution of compound 11 (200 mg, 0.48 mM) in dry THF (3.5 mL) was added dropwise via a syringe n-tetrabutylammonium fluoride (1 M solution in THF, 1.8 mL, 1.8 mM, 3.75 eq).[21] The reaction mixture was stirred for 24 h after which time the solvent was removed under reduced pressure. Purification of the crude reaction mixture using flash chromatography (SiO_2 : 25 g, elution first with hexanes then with hexanes-ethyl acetate) afforded pure compound 13 as a crystalline material (51 mg, 61 %). Recrystallization from hexanes afforded compound 13 as white fine needles (40 mg), mp 75°C ; $[\alpha]_D^{23}$ = + 101° (c 0.40, Chloroform) ; ^{1}H NMR (Table I) ; IR (KBr) : 3560-3300 and 3280-3120 (broad, OH) ; MS (C_8 H_{16} O_4, M.W. = 176) : 194 (11) $(M + NH_4)^+$, 162 (100) $(M + NH_4 - CH_3OH)^+$.

Anal. Calcd for C_8 H_{16} O_4 : C, 54.53 ; H, 9,15. Found : C, 54.42 ; H, 8.97.

Methyl 2,6-dideoxy-4-O-methyl-α-L-arabino-hexopyranoside[22,23] 14

Pure L-enantiomer 14 was obtained in a similar way[21] from 12 (566.5 mg, 1.37 mM) as a crystalline material (159 mg, 67.5 %). Recrystallization form hexanes afforded compound 14 as white fine needles (106 mg), mp 76-78°C ;$[\alpha]_D^{23}$ = - 139° (c 0.45, Chloroform); (lit.[22] mp 70-72°C,$[\alpha]_D^{23}$ = - 132.7 (c 0.90, Chloroform ; lit.[23] mp 75-77°C (from hexanes-acetone), $[\alpha]_D^{23}$ = -105°) ; ^{1}H NMR (Table I); IR (KBr) : 3560-3300 and 3280-3120 (broad, OH) ; MS ($C_8H_{16}O_4$, M.W. = 176) : 194 (10), 162 (100).

Serological properties

50 μL of an ethanolic solution of Phe Gl K-I (2,5 μg/mL) were deposited in wells of a microtiter plate (Nunc immuno-plate I 96 F - Nunc - Denmark) and allowed to dry at 37°C. The wells were then saturated with 100 μL of PBS + 5 % BSA and left 2 h at 37°C in a moist chamber. The wells were rinsed and then filled with 90 μL of the inhibiting sugar solution (see text) and 10 μL of rabbit polyclonal anti Phe Gl K-I serum diluted 1/10 in PBS + 1 % BSA. The plate was left overnight at 4°C, and rinsed with PBS + 1 % BSA three times.

100μL of the conjugate solution (anti rabbit Ig-β-Galactosidase linked whole antibody from donkey, Amersham, France) diluted 1/750 were left 2 h at 37°C in a moist chamber and then rinsed three times. The substrate was added (100 μL/well) and left one h at 37°C. The absorbances at 405 nm were read with an ELISA plate reader spectrophotometer Titerteck multiscan plus (FLOW labs, Puteaux, France). The results were presented on a Logistic calibration mode using the Flow labs software.

The blank value was averaged from 8 wells in which all the procedure was performed without initial antigen coating. The 100 % positive reaction was averaged from 12 wells in which no inhibiting sugar solution (i.e. 90 μL PBS + 1 % BSA) was added. Each value was obtained in triplicate.

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REFERENCES AND FOOTNOTES

1. J.H. Bates in "The Mycobacteria A Sourcebook", vol. B ; G.P. Kubica and L.G. Wayne, Eds. ; Marcel Dekker, Inc., New York, 1984, p. 991.

2. C.O. Thoen, A.G. Karlson and E.H. Himes in "The Mycobacteria A Sourcebook", vol. B ; G.P. Kubica and L.G. Wayne, Eds. ; Marcel Dekker, Inc., New York, 1984, p. 1209.

3. A.M. Lowell in "The Mycobacteria a Source book", vol. B ; G.P. Kubica and L.G. Wayne, Eds., Marcel Dekker, Inc. New York, 1984, p. 1021.

4. T. Godal and L. Levy in "The Mycobacteria A Sourcebook", vol. B ; G.P. Kubica and L.G. Wayne, Eds. ; Marcel Dekker, Inc., New York, 1984, p. 1083.

5. E. Wolinsky in "The Mycobacteria A Sourcebook", vol. B ; G.P. Kubica and L.G. Wayne, Eds. ; Marcel Dekker, Inc., New York, 1984, p. 1141.

6. F.M. Collins, Int. J. of Leprosy, 54, 458 (1986).

7. P.J. Brennan and M.B. Goren, J. Biol. Chem., 254, 4205 (1979).

8. P.J. Brennan, Microbiology, 53, 366 (1984).

9. J.J. Fournié, M. Rivière and G. Puzo, J. Biol. Chem., 262, 3174 (1987).

10. S.W. Hunter, I. Jardine, D.L. Yanagihara and P.J. Brennan, Biochemistry, 24, 2798 (1985).

11. J.J. Fournié, M. Rivière, F. Papa and G. Puzo, J. Biol. Chem., 262, 3180 (1987).

12. J.J. Fournié, M. Rivière and G. Puzo, Eur. J. Biochem., 168, 181 (1987).

13. B. Fraser-Reid, D.R. Kelly, D.B. Tulshian and P.S. Ravi, J. Carbohydr. Chem., 2, 105 (1983).

14. F. Sztaricskai, I. Pelyvas, L. Szilagyi, R. Bognar, J. Tamas and A. Neszmelyi, Carbohydr. Res., 65, 193 (1978).

15. W. Roth and W. Pigman, Methods Carbohydr. Chem., Vol. II, 405 (1963) ; L. Goodman, E.M. Acton, C.W. Mosher and P. Marsh, Methods Carbohydr. Chem., Vol. VIII, 207 (1980).

16. P.L. Durette, Carbohydr. Res., 100, C27 (1982).

17. J.P. Marsh, Jr., C.W. Mosher, E.M. Acton and L. Goodman, J. Chem. Soc., Chem. Commun., 973 (1967).

18. S. Hanessian and P. Lavallée, Can. J. Chem., 53, 2975 (1975).

19. J. Arnarp, L. Kenne, B. Lindberg and J. Lonngren, Carbohydr. Res., 44, C5 (1975).

20. J.M. Berry and L.D. Hall, Carbohydr. Res., 47, 307 (1976).

21. E.J. Corey and A. Venkateswarlu, J. Am. Chem. Soc., 94, 6190 (1972).

22. J. Yoshimura, M. Matsuzawa, K.I. Sato and Y. Nagasawa, Carbohydr. Res., 76, 67 (1979).

23. C. Monneret, R. Gagnet and J.C. Florent, J. Carbohydr. Chem., 6, 221 (1987).

24. G. Grethe, T. Mitt, T.H. Williams and M.R. Uskokovie, J. Org. Chem., 48, 5309 (1983).

25. P. Bartner, D.L. Boxler, R. Brambilla, A.K. Mallams, J.B. Morton, P. Reichert, F.D. Sancilio, H. Surprenant, G. Tomalesky, G. Lukacs, A. Olesker, T. That Thang, L. Valente, S. Omure, J. Chem. Soc. Perkin I, 1600 (1979).

26. F. Papa, M. Rivière, J.J. Fournié, G. Puzo and H. David, J. Clin. Microbiol., 25, 2270 (1987).

27. T. Fujiwara, S.W. Hunter, S.N. Cho, G.O. Aspinall and P.J. Brennan, Infect. Immun., 43, 245 (1984).

28. S.J. Brett, S.N. Payne, P. Draper and R. Gigg, Clin. Exp. Immunol., 56, 89 (1984).

29. J. Stanek, Jr., M. Marek and J. Jary, Carbohydr. Res., 64, 315 (1978) and references cited herein.

30. P.L. Durette, Synthesis, 12, 1037 (1980).

31. M.R. Winkle, J.M. Lansinger and R.C. Ronald, J. Chem. Soc., Chem. Commun., 87 (1980).