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CHEMICAL SYNTHESIS OF AN ARTIFICAL ANTIGEN CONTAINING THE TRISACCHARIDE HAPTEN OF *Mycobacterium leprae*

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ABSTRACT

The trisaccharide allyl *O*-(3,4-di-*O*-methyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-methyl-α-L-rhamnopyranosyl)-(1→2)-3-*O*-methyl-α-L-rhamnopyranoside was synthesized from partially methylated monosaccharide derivatives. Condensation of 1,4-di-*O*-acetyl-2,3-di-*O*-methyl-α-L-rhamnopyranose promoted by boron trifluoride etherate with the appropriate alcohol proceeded stereoselectively and with very high yields. Selective deacetylation and glycosylation with 2,4-di-*O*-acetyl-3,6-di-*O*-methyl-α-D-glucopyranosyl bromide led to a trisaccharide. The acrylamide copolymers of mono-, di-, and tri-saccharide were compared in their ability to specifically bind antibodies from leprosy patients.

INTRODUCTION

The detection of antibodies toward the oligosaccharide component of the phenolic glycolipid of *Mycobacterium leprae* is of great value for the early diagnosis of leprosy[1-3]. The trisaccharide *O*-(3,6-di-*O*-methyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-methyl-α-L-rhamnopyranosyl)-(1→2)-3-*O*-methyl-α-L-rhamnopyranoside is also implicated in the activation of T-supressor cells[4] and is a suitable target for the production of specific poly- and mono-clonal antibodies. In our previous paper[5], we described the synthesis of the terminal nonreducing disaccharide as the allyl glycoside. We report now the synthesis of the trisaccharide as the allyl glycoside and the use of acrylamide copolymers of mono-, di-, and tri-saccharide in the detection of antibodies.

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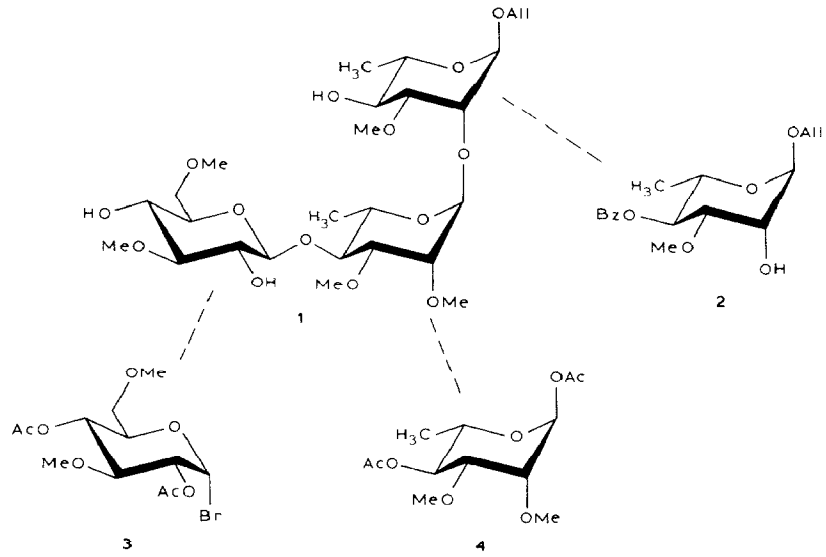
RESULTS AND DISCUSSION

Our synthesis of trisaccharide **1** differs from those previously reported[6,7] in that the partially methylated synthons **2**, **3**, and **4** were used to avoid benzyl protecting groups. This was essential in order to obtain the final product as an allyl glycoside. 4-Methoxybenzyl ethers have also been used in place of benzyl ethers to obtain allyl glycosides[8]. Compound **2** was obtained from L-rhamnose in seven steps. The monosaccharide was acetylated with acetic anhydride–perchloric acid and then glycosidated with allyl alcohol and stannic chloride[9]. In our hands, the yield and purity of allyl L-rhamnoside thus obtained was higher than that previously reported with Fischer's method[10]. After deacetylation and reaction with acetone–4-toluenesulfonic acid, allyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**5**) was obtained in a 53% yield. Benzoylation, followed by hydrolysis with trifluoroacetic acid at room

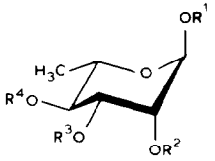


temperature) gave a diol **6** in 95% yield. This was treated with methyl iodide and dibutyltin oxide to yield **2** (yield 86%), the ^{13}C-n.m.r. spectrum of which was in accordance with the proposed structure.

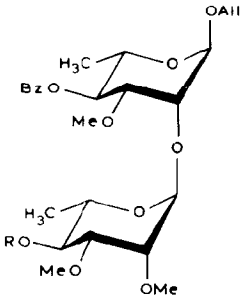
The second L-rhamnose compound **4** was obtained from benzyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside[11] which was benzylated with benzyl chloride–NaH in *N*,*N*-dimethylformamide to give **7** in 75% yield. The ^1H-n.m.r. spectrum was of first order and agreed well with the proposed structure. Hydrolysis with

hydrochloric acid in ethanol produced diol **8** in 80% yield and methylation with methyl iodide–sodium hydride in *N,N*-dimethyl formamide gave **9** which was hydrogenolyzed with palladium-charcoal. Conventional acetylation gave **4** in a 59% overall yield from **8**.

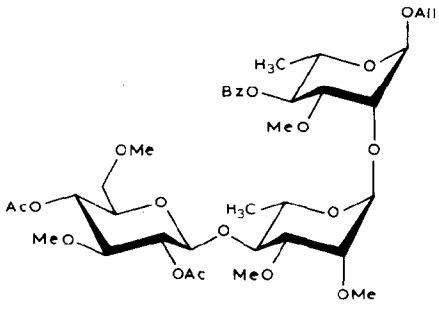
The use of glycosyl donor of type **4**, which is methylated at O-2, should be conducted with caution. In the first synthesis of this trisaccharide reported by Fujiwara *et al.*,[6] a similar reaction with a glycosyl bromide having a nonparticipating group at O-2 gave a mixture of α and β anomers of the trisaccharide. In the *manno (rhamno)* series, the formation of α anomer is specially favored thermodynamically. Thus, we used a Lewis acid-catalyzed glycosidation that mostly proceed *via* SN1 mechanism. This coupling reaction was carried out directly with diacetate **4** and allyl glycoside **2** in the presence of boron trifluoride etherate to give, in 87% yield, disaccharide **10**. The configuration of the anomeric carbon atom was ascertained by $J_{C\text{-}1,H\text{-}1}$ 170.5 Hz for δ 98.3. This value (> 165 Hz) is characteristic of the α-L-rham-



5 R^1 = All, R^2 = R^3 = Me_2C, R^4 = H
6 R^1 = All, R^2 = R^3 = H, R^4 = Bz
7 R^1 = R^4 = Bn; R^2, R^3 = Me_2C
8 R^1 = R^4 = Bn, R^2 = R^3 = H
9 R^1 = R^4 = Bn, R^2 = R^3 = Me



10 R = Ac
11 R = H



12 R^1 = Bz, R^2 = Ac

All = CH_2—CH=CH_2

noside bond[12]. Selective deacetylation[13] with HCl in methanol–dichloromethane gave disaccharide **11** having OH-4′ free in 90% yield.

Glycosidation of this disaccharide with 2,4-di-*O*-acetyl-3,6-di-*O*-methyl-α-D-glucopyranosyl bromide[5] (**3**) in acetonitrile in the presence of mercury cyanide gave trisaccharide **12** in 68% yield. This was deacylated with M sodium hydroxide and the allyl glycoside trisaccharide **1** was obtained in 85% yield. The ^{1}H- and ^{13}C-n.m.r. spectra confirmed the proposed structure.

The previous studies[6,14] with a synthetic conjugate showed that a disaccharide is the minimal structure required for an effective binding with antibodies. This was

confirmed recently[15–17], but in many cases the terminal monosaccharide displayed the same capacity.

We synthesized the mono-[18], di-[5], and tri-saccharide described herein as allyl glycosides, because this group is a versatile tool that may be used for coupling with BSA after ozonolysis[19], for elongation, or for copolymerization with acrylamide. The latter procedure was applied to all the aforementioned compounds by published methods[20,21]. Copolymers with a mol wt. of 100 000 and a carbohydrate content of 27% were obtained; they had the advantage that carbohydrate incorporation into the antigens was higher than with those obtained by coupling to proteins. A preliminary study of these copolymers in the ELISA test against IgM from pooled sera of leprosy patients and healthy donors is given in Table I. The three copolymers showed high activity, thus confirming that the terminal monosaccharide unit is the combining site for a majority of the antibodies. A comparison of the activities of copolymers with those of glycoconjugates in the ELISA test has been published[22]. The sensitivity of both series of compounds was similar, but the selectivity of the copolymers was somewhat poorer.

EXPERIMENTAL

General methods. — Melting points were determined with a Boethius apparatus and are uncorrected. Optical rotations were measured at 22–25° with a Polamat A polarimeter for 1% solutions in chloroform unless otherwise stated. ^{1}H- and ^{13}C-n.m.r. spectra were recorded with a Jeol JNM-FX90Q spectrometer for solutions in $CDCl_3$. Chemical shifts are given relative to the signal of tetramethylsilane (δ 0.0). The ^{1}H- and ^{13}C-n.m.r. spectra of trisaccharide **1** were recorded with a Bruker 500 spectrometer for solutions in $CDCl_3$ by Dr. K. Bock as a part of an HSEA-n.m.r. study of this molecule. Assignments were based on COSY experiments. The pro-

TABLE I

COMPARISON OF ACTIVITIES AGAINST POOLED LEPROSY AND NORMAL SERA OF SYNTHETIC COPOLYMERS

Acrylamide copolymer	*Mean + SD A_{492} for sera of*	
of	*Leprosy patients(12)*	*Normal humans (30)*
3,6-Di-*O*-methyl-β-D-glucopyranosyl	1.178 ± 1.00	0.027 ± 0.004
O-(3,6-Di-*O*-methyl-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-methyl-α-L-rhamnopyranosyl	1.450 ± 1.03	0.043 ± 0.006
O-(3,6-Di-*O*-methyl-β-D-glucopyranosyl-(1→4)-*O*-(2,3-di-*O*-methyl-α-L-rhamnopyranosyl)-(1→2)-3-*O*-methyl-α-L-rhamnopyranosyl	1.225 ± 0.943	0.026 ± 0.003

gress of reactions was monitored by t.l.c. on Silica Gel G(Merck). Flash chromatography was performed on Silica Gel G. The solvent systems are indicated by volume-to-volume ratios. Components were detected by spraying the plates with 20% conc. H_2SO_4 and heating or with a solution containing 1% of $KMnO_4$ in 2% Na_2CO_3 for compounds containing the allyl group. Elemental analyses were performed by Dr. Odila Olivares at the Institute of Chemistry. The ELISA test was performed according to Cho *et al.*[14], except that PVC microtiter plates were used. A more detailed description of this study will be publised separately.

Allyl 4-O-benzoyl-3-O-methyl-α-L-rhamnopyranoside (**2**). — Allyl 4-*O*-benzoyl-α-L-rhamnopyranoside[5] (**6**, 2 g) and dibutyltin oxide (2.2 g) were heated under reflux with benzene (50 mL) with continuous azeotropic removal of water. After 2 h, the solvent was evaporated, the residue was dissolved in methyl iodide (20 mL), and the solution was stirred overnight at 35–40°. The solution was evaporated and the residue was purified by column chromatography in 10:1 chloroform–acetone. Compound **2** crystallized from ethanol, yield 1.8 g (86%), m.p. 101–104°, $[\alpha]_D^{25}$ −57°; ^{13}C-n.m.r.: δ 165.9(CO) 133.8(CH) 133.1, 129.7,128.4 (PhCO), 117.7 (CH), 98.1 (C-1), 79.1 (C-3), 73.3 (C-4), 68.3 (OCH_2), 67.9, 66.3 (C-2 and C-5), 57.9 (MeO), and 17.4 (C-6). No satisfactory analysis could be obtained for this compound because of contamination with organotin residue.

Benzyl 4-O-benzyl-2,3-O-isopropylidene-α-L-rhamnopyranoside (**7**).— To a solution of benzyl 2,3 - *O* - isopropylidene - α - L - rhamnopyranoside[23] (1 g) in *N,N*-dimethylformamide (12 mL), was added NaH (500 mg), and the mixture was stirred for 30 min. After cooling at 0°, benzyl chloride (0.56 mL) was added dropwise, and stirring was continued overnight. Methanol (2 mL) was added dropwise, and the mixture was added to ice-water. The precipitate was filtered off, dried, and recrystallized from ethanol, yield 0.98 g (75%), m.p. 89–90°, $[\alpha]_D^{25}$ −64°; ^{1}H-n.m.r.: δ 7.23 (m, 10 H, Ph), 5.06 (s, 1 H, H-1) 4.70 (dd, $C_6H_5CH_2$) 3.78 (dd, 1 H, $J_{6,5}$ 9.7 Hz, H-5), 3.24 (dd, 1 H, $J_{6,7}$ 9.7 Hz, H-4), 4.18 (d, 1 H, *J* 5.5 Hz, H-2), 4.30 (dd, 1 $J_{6,7}$ 5.5 Hz, H-3), 1.51 (s, 3 H, Me), 1.29 (s, 3 H, Me), and 1.20 (d, 3 H, H_3-6).

Anal. Calc. for $C_{23}H_{28}O_5$: C, 71.85; H, 7.34. Found: C, 71.60; H, 7.21.

Benzyl 4-O-benzyl-α-L-rhamnopyranoside (**8**). — Compound **7** (700 mg) was dissolved in ethanol (5 mL), and 25mM HCl in ethanol was added. The solution was boiled under reflux for 1 h. After neutralization with an $NaHCO_3$ solution, the solvent was evaporated. The residue was dissolved in chloroform, washed with water, dried, and evaporated, yield 80%, m.p. 85–86°, $[\alpha]_D^{25}$ −57° (ethyl acetate); ^{1}H-n.m.r.: δ 7.36 (m, 10 H, Ph) 4.88 (s, 1 H, H-1), 3.98 (m, 2 H, H-2,3), 3.09 (s,OH), 2.89 (s, OH), and 1.40 (d, 3 H, H_3-6); lit.[10,23] m.p. 85–86°, 86–88°; $[\alpha]_D$ −93°.

Benzyl 4-O-benzyl-2,3-di-O-methyl-α-L-rhamnopyranoside (**9**). — Compound **8** (1 g) was dissolved in *N,N*-dimethylformamide (18 mL). NaH (500 mg) was added, and the mixture was stirred for 30 min. After cooling to 0°, methyl iodide (2.5 mL) was added dropwise and stirring was continued overnight at room temperature.

Methanol was added and the mixture was diluted with chloroform, washed with water, dried, and evaporated. *N,N*-Dimethylformamide was evaporated in high vacuum, yield 1.08 g (100%), $[\alpha]_D^{25}$ −59° ^{1}H-n.m.r.: 7.28 (m,10 H,Ph), 4.91 (s, 1 H,H-1) 4.55 (dd, 4 H, $C_6H_5CH_2$), and 1.31 (d, 3 H,H_3-6).

Anal. Calc. for $C_{22}H_{28}O_5$; C, 70.94; H, 7.58. Found: C, 70.96; H, 7.42.

1,4-Di-O-acetyl-2,3-di-O-methyl-α-L-rhamnopyranose (**4**). — Compound **9** (750 mg) was hydrogenolyzed in 1:1 (v/v) ethyl acetate–acetic acid in the presence of 5% Pd-C. After all the compound had reacted, the mixture was filtered, and the filtrate was evaporated to give quantitatively 2,3-di-*O*-methyl-L-rhamnose. This was acetylated with pyridine–acetic anhydride to give **4**, yield 556 mg (100%), amorphous, $[\alpha]_D^{25}$ −41°; ^{1}H-n.m.r.: δ 6.15 (s, 1 H, H-1), 5.03 (m, 1 H, H-4), 3.54 (s, OMe), 3.45 (s, OMe), 2.14 (s, 3 H, Ac), 2.10 (s, 3 H, Ac), and 1.18 (d, 3 H, H_3-6).

Anal. Calc. for $C_{12}H_{20}O_7$: C, 52.16; H, 7.30. Found: C, 52.24; H, 7.30.

Allyl O-(4-O-acetyl-2,3-di-O-methyl-α-L-rhamnopyranosyl)-(1→2)-4-O-benzoyl-3-O-methyl-α-L-rhamnopyranoside (**10**). — The acetate 4 (87.5 mg) and the alcohol **2**, (70 mg) were dried in a high vacuum for 4 h, and dichloromethane (stored in vacuum over CaH_2) was distilled directly into the reaction vessel, which was then disconnected from the vacuum line under positive Ar pressure. The mixture was cooled and boron trifluoride etherate (11.6 μL) was added. Stirring was continued overnight at room temperature, and the mixture was diluted with dichloromethane, washed with an $NaHCO_3$ solution, dried, and evaporated. Column chromatography (20:1, v/v, chloroform–acetone) afforded **10** (102 mg, 87%), $[\alpha]_D^{25}$ −35°; ^{13}C-n.m.r.: δ 170.0, 165.9 (C-O); 133.6(C=H), 133.2, 130.0, 129.7, 128.5 (PhCO), 117.8 (CH_2), 99.5 (C-1), 98.3 (C-1′), 79.2 (C-3), 78.7, 76.7 (C-2′,3′) 74.1(C-2), 73.7, 73.0 (C-4, 4′), 68.2 (OCH_2), 67.2, 67.0 (C-5,5′), 59.3, 58.5, 57.9 (MeO), 21.0 (Ac), and 17.6 (C-6).

Anal. Calc. for $C_{27}H_{38}O_{11}$: C, 60.21; H, 7.11. Found: C, 60.64; H, 7.14.

Allyl O-(2,3-di-O-methyl-α-L-rhamnopyranosyl)-(1→2)-4-O-benzoyl-3-O-methyl-α-L-rhamnopyranoside (**11**). — To a solution of disaccharide **10** (200 mg) in dichloromethane (0.64 mL) was added a solution of HCl in methanol (1.6 mL; prepared by adding 0.2 mL of acetyl chloride to 5 mL of dry methanol at 0°). The solution was stirred for 20 h, diluted with dichloromethane, washed with Na_2CO_3 solution, with water, dried, and evaporated. Purification by column chromatography (30:1, v/v, chloroform–acetone) afforded **11** (165 mg, 89%), $[\alpha]_D^{25}$ −38°.

Anal. Calc. for $C_{25}H_{36}O_{10}$: C, 60.47; H, 7.31. Found: C, 60.56; H 7.64.

Allyl O-(2,4-di-O-acetyl-3,6-di-O-methyl-β-D-glucopyranosyl)-(1→4)-O-(2,3-di-O-methyl-α-L-rhamnopyranosyl)-(1→2)-4-O-benzoyl-3-O-methyl-α-L-rhamnopyranoside (**12**).—Glycosylation of disaccharide **11** (160 mg) with 2,4-di-*O*-acetyl-3,6-di-*O*-methyl-α-D-glucopyranosyl bromide[5] (**3**; (180 mg) and $Hg(CN)_2$ (92 mg) was performed in acetonitrile as described previously[5]; yield 162 mg (68%), $[\alpha]_D^{25}$ −38°; ^{13}C-n.m.r.: δ 169.6, 169.3, 165.9 (C=O), 133.1(CH=), 129.9, 129.6, 128.4 (PhCO), 117.8 (CH_2=), 100.9 ($J_{C,H}$ 163.7 Hz, C-1″), 98.2 $J_{C,H}$ 172.4 Hz, (C-1,1′), 81.5, 80.8, 79.6, 75.7, 73.7, 72.9, 72.9, 72.7, 72.3, 72.0, 70.2, 68.1, 66.7, 66.9, 69.7

(C-2,3,4,5,6), 58.9, 58.5, 58.3, 57.3, 57.1 (5 MeO), 21.1, 20.9 (Ac), and 17.8, 17.5(C-6,6').

Anal. Calc. for $C_{37}H_{54}O_{17}$: C, 57.65; H, 7.06. Found: C, 57.74; H, 7.28.

Allyl O-(3,6-di-O-methyl-β-D-glucopyranosyl)-(1→4)-O-(2,3-di-O-methyl-α-L-rhamnopyranosyl)-(1→2)-3-O-methyl-α-L-rhamnopyranoside (**1**). — Trisaccharide **12** was dissolved in methanol (2 mL), 1.25M NaOH solution (2 mL) was added, and the mixture was heated to 60° for 6 h. The solution was cooled, extracted with dichloromethane (2 x 10 mL), dried, and evaporated to give a chromtographically homogeneous compound (78 mg, 85%), $[\alpha]_D^{25}$ −35°; ^{1}H-n.m.r. (500 MHz): δ 5.90(m, 1 H, CH=), 5.292 (m, 1 H, CH_2=), 5.216 (m, 1 H, CH_2=), 5.059 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.775(d, 1 H, $J_{1',2'}$ 1.6 Hz, H-1), 4.407(d, 1 H, $J_{1''2''}$ 8.0 Hz, H-1''), 4.074 (dd, 1 H, $J_{2'3'}$ 3.5 Hz, H-2'), 3.703 (dd, 1 H, $J_{2,3}$ 3.5 Hz, H-2), 3.675 (s, 3 H, MeO), 3.525 (dd, 1 H, $J_{3''4''}$ 9.6 Hz, H-4''), 3.483, 3.470, 3.463, 3.390(4s, 12 H, 4 MeO); 3.159 (dd, 1 H, $J_{2''3''}$ 9.2 Hz, H-3''), 1.333 (d,3 H, H_3-6), and 1.307(d, 3 H, H_3-6'), ^{13}C-n.m.r. (125 MHz): δ 133.547 (CH=), 117.504 (CH_2=) 105.480 (C-1''β), 98.094 (C-1'), 98.042 (C-1), 85.412 (C-3''), 81.561, 81.502, 80.059, 75.664, 74.892 (C-2,3,2',3',4'), 73.918, 71.780, and 71.738. (*C*-4,2'',4''), 72.725 (C-6''), 71.084 (C-5''), 68.232 (C-5'), 68.010 (C-5'), 67.717 (OCH_2), 60.362 (MeO-6''), 59.464 (MeO-3''), 58.892, 56.356 (MeO-2',3'), 57.358 (MeO-3), 17.596, 17.424 (C-6,6').

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