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Synthesis of α,α-trehalose 2,3- and 2,3′-diesters with palmitic and stearic acid: potential immunoreactants for the serodiagnosis of tuberculosis

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ABSTRACT

Regioselective monoacylation, by the stannylation method, of 4,6:4′,6′-di-*O*-benzylidene-α,α-trehalose with palmitoyl or stearoyl chloride afforded the 2-palmitate and 2-stearate of the diacetal, whereas partial diacylation led to the corresponding 2,3′-dipalmitate and 2,3′-distearate. Protection of the monoesters in the 2′,3′ positions by cyclizing silylation with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, followed by acylation of the silyl ethers, gave the fully protected 2,3-dipalmitate, 2,3-distearate, and 2-palmitate-3-stearate. Small proportions of other isomers and triesters were also produced in these reactions. Desilylation and debenzylidenation of the diesters finally furnished 2,3- and 2,3′-di-*O*-palmitoyl-, 2,3- and 2,3′-di-*O*-stearoyl-, and 2-*O*-palmitoyl-3-*O*-stearoyl-α,α-trehalose.

INTRODUCTION

Mycobacteria produce a large variety of glycolipids that contain α,α-trehalose (**1**) as the carbohydrate core. Among them are the cord factors (6,6′-dimycolates of **1**), as well as numerous derivatives in which **1** is esterified in other positions and by other fatty acids. Some of these compounds are sulfatides (sulfolipids), bearing a sulfate substituent in position 2 of one of the glucose units. The attached fatty acids may be of manifold structural types, including simple, relatively low molecular weight alkanoic acids (e.g., palmitic and stearic acid) as well as longer chain, multimethyl-branched alkanoic, alkenoic, and hydroxyalkanoic acids (e.g., phthioceranic and hydroxyphthioceranic acids, mycolipenic [phthienoic] and mycolipanolic acids, and others) having chain lengths of up to 40 carbon atoms[1].

In 1985, Minnikin et al.[2] described a family of novel, closely related trehalose-

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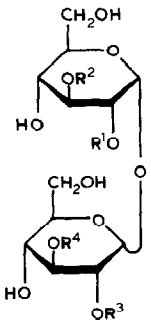
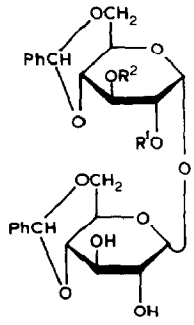
based glycolipids with antigenic activity, which were isolated from tubercle bacilli (*M. tuberculosis* strains C and H37Rv) and shown to contain C_{14}–C_{24} alkanoic, alkenoic, and 10-methylalkanoic acids (principally palmitic, stearic, palmitoleic, oleic, tuberculopalmitic, and tuberculostearic acids), 2,4-dimethyldocosanoic acid, and higher acids of the mycolipenic and mycolipanolic type (principally, 2,4,6-trimethyltetracos-2-enoic and 3-hydroxy-2,4,6-trimethyltetracosanoic acids). The distribution of the acyl groups on the trehalose molecule was not investigated at the time. Subsequently, Papa et al.[3] isolated from a number of *M. tuberculosis* strains a glycolipid, minor in proportion but highly antigenic and promising for use in the serodiagnosis of tuberculosis and leprosy[4], which they originally believed to be a sulfatide related to previously encounterd, mycobacterial sulfatides (SL-I, SL-II, SL-III)[1,5] and therefore termed[4] SL-IV. The structure of a 2,3-di-*O*-acyltrehalose 2′-sulfate was tentatively assigned, with the acyl groups comprising chiefly palmitoyl and stearoyl[3c]. More detailed examinations[6] of the structure confirmed the positions (O-2 and O-3) of the lipid ester groups; however, they revealed that sulfate is absent and that palmitic and stearic acid represent but a modest proportion of the overall acid complement which proved much more complex, comprising essentially the same components as those found in Minnikin's[2] glycolipids, with a large proportion of higher acids (mainly C_{24}–C_{27}). In light of these results it appeared of interest to determine whether or to what extent the higher acids are necessary for the expression of antigenicity, and we therefore decided to synthesize a number of di-*O*-acyltrehaloses (**2**–**7**) containing palmitoyl and (or) stearoyl groups only, in positions 2 and 3 of the glucose rings, for eventual study of their antigenic properties.

RESULTS AND DISCUSSION

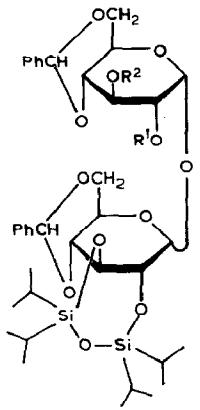
α,α-Trehalose 2,3-dipalmitate (**2**) was selected as the first target. Readily available[7] 4,6:4′,6′-di-*O*-benzylidene-α,α-trehalose (**8**) was treated with 1.1 molar equivalents of dibutyltin oxide in boiling methanol to form a 2,3-*O*-dibutylstannylene derivative, which was treated in situ with 1.2 molar equivalents of palmitoyl chloride in the presence of triethylamine. Acylation occurred mainly at the activated O-2 position[8], with replacement of the stannylene group, to furnish a 66% yield of the 2-palmitate **9** after chromatographic removal of traces of less polar, unidentified by-products. The ^{1}H NMR spectrum of isolated **9** (dd at δ 4.88 for H-2) showed no evidence for contamination by the isomeric 3-palmitate (expected to give a characteristic triplet near δ 5.60). Compound **9** was then protected at O-2′,3′ by cyclizing silylation[9] with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine, performed for 2 days at room temperature. The major product seen in TLC was separated chromatographically from several minor sideproducts and found to be the expected silyl derivative **15** (yield, 40%). One of the minor products, isolated in 3% yield, was the 3-*O*-palmitoyl isomer **16** which appears to have resulted from acyl migration during the process since the starting



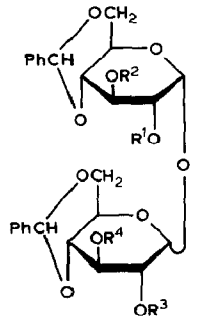
	R^1	R^2	R^3	R^4
1	H	H	H	H
2	Pa	Pa	H	H
3	Pa	St	H	H
(**4**	St	Pa	H	H)
5	St	St	H	H
6	St	H	H	St
7	Pa	H	H	Pa



	R^1	R^2
8	H	H
9	Pa	H
10	St	H
11	Pa	Pa
12	Pa	St
13	St	Pa
14	St	St



	R^1	R^2
15	P	H
16	H	Pa
17	Pa	Pa
18	Pa	St
19	St	Pa
20	St	H
21	H	St
22	St	St



	R^1	R^2	R^3	R^4
23	St	H	H	St
24	Pa	H	H	Pa
25	H	Pa	H	Pa
26	St	St	H	St
27	St	St	St	H
28	Pa	H	BrAc	H
29	Pa	St	BrAc	St
30	Pa	St	H	St

BrAc = $BrCH_2CO$
Pa = $CH_3(CH_2)_{14}CO$
St = $CH_3(CH_2)_{16}CO$

compound **9** had been isomerically pure. Palmitoylation of **15** (usable in crude form containing some **16**) furnished the fully blocked 2,3-dipalmitate **17** in 83% yield. This was desilylated by tetrabutylammonium fluoride to give the crystalline 2′,3′-diol **11** (89%), *O*-debenzylidenation of which, with iodine in methanol[10], then afforded **2** (89%).

Stearoylation of (pure) **15**, followed by sequential desilylation and deacetalation by the methods just mentioned, provided the corresponding 2-*O*-palmitoyl-3-*O*-stearoyl derivatives **18**, **12**, and **3** in excellent yields. The small amount of available 3-palmitate **16** was similarly stearoylated, to give the 3-*O*-palmitoyl-2-*O*-stearoyl derivative **19**, a regioisomer of **18**; it was desilylated to **13**, but lack of material precluded further transformation into the fully deprotected diester **4**.

Next, the just-described procedure for the monoacylation of **8** was performed with stearoyl chloride to produce the 2-stearate **10**, isolated after chromatography in 51% yield. In this instance, less-polar by-products (TLC) arose in larger proportions than in the palmitoylation, and two of these were isolated. The least polar material, obtained in 4% yield, was shown by NMR spectroscopy and microanalysis to be a mixture of tristearates (even though it gave a single spot in TLC). One component of that mixture was almost certainly the 2,3,3′-tristearate **26**, as there was a set of ^{1}H NMR signals matching exactly those of independently synthesized 2-*O*-palmitoyl-3,3′-di-*O*-stearoyl derivative **30** which was at hand for comparison. (Replacement of stearoyl by palmitoyl in such esters generally entails no significant spectral differences; see a subsequent paragraph.) Other signals present in the spectrum of the mixture were attributable to the isomeric 2,3,2′-tristearate **27**. The second by-product, isolated in substantial quantity (25%), was revealed by its ^{1}H NMR spectrum to be the 2,3′-distearate **23**; upon debenzylidenation by iodine in methanol it yielded crystalline α,α-trehalose 2,3′-distearate (**6**, 78%).

Stannylenation of **8** as already described, followed by treatment with 2 molar equivalents of palmitoyl chloride, gave as the main product the 2,3′-dipalmitate **24**, isolated crystalline in 35% yield by chromatography, which also produced a small proportion of isomeric 3,3′-dipalmitate **25** and a trace amount of what appeared to be a mixture of tripalmitates (NMR). Deacetalation of **24** with trifluoroacetic acid furnished crystalline α,α-trehalose 2,3′-dipalmitate (**7**); the yield (45%) was inferior to those obtained in the aforementioned deacetalations using iodine.

For the preparation of α,α-trehalose 2,3-distearate (**5**), the 2-stearate diacetal **10** was subjected to cyclizing silylation as described for **9**. Inexplicably, the silylating agent reacted with **10** more sluggishly than it had with **9**; at room temperature, no reaction was observed, and after overnight heating at 50–60 °C a yield of only 31% of pure silyl ether **20** (R_f 0.70) was obtained following chromatography of the reaction mixture which contained several by-products. One major chromatographic fraction (34%) was a mixture of **20** with its 3-*O*-stearoyl isomer **21** (R_f 0.73), resulting from acyl migration. Fast-moving by-products that gave two spots in TLC (R_f 0.82–0.85) emerged unseparated from the preparative

column, amounting to 16% of the isolated material. The precise nature of these by-products could not be established, but the ^{1}H NMR spectrum indicated the presence of more than four isopropyl groups and hence, of more than one –*O*–Si $(CHMe_2)_2$–O–Si $(CHMe_2)_2$–O– grouping, introduced perhaps in part in noncyclizing fashion *.

Stearoylation of the mixture of monostearates **20** and **21** (or of pure **20**) gave ~90% yields of the corresponding 2,3-di-*O*-stearoyl derivative **22**, which was desilylated to the diol **14** (86%) **. Deacetalation (I_2/MeOH) of **14** afforded crystalline **5** (82%).

In summary, the 2,3- and 2,3′-dipalmitates (**2** and **7**), the 2,3- and 2,3′-distearates (**5** and **6**), and the 2-palmitate-3-stearate (**3**) of α,α-trehalose were synthesized. The 3-palmitate-2-stearate and the 3,3′-dipalmitate were obtained as their 4,6:4′,6′-di-*O*-benzylidene derivatives (**13** and **25**) in small amounts.

The 2,3,3′-triester **30**, used for spectral comparison, was prepared on a small scale without full characterization of intermediates. Thus, the 2-palmitate **9** was acylated with bromoacetyl bromide by the stannylene procedure, to give the 2′-*O*-bromoacetyl-2-*O*-palmitoyl derivative **28**, which was then stearoylated at O-3,3′. Selective cleavage of the bromoacetyl group in the resulting ester **29** with thiourea[11] furnished **30**.

Nuclear magnetic resonance spectra.—The ^{1}H NMR spectra of all trehalose esters showed the same, familiar pattern of resonances for the alkanoyl groups, namely, a triplet at δ 0.85 for terminal CH_3, a large unresolved peak centered at δ 1.23 for chain-internal CH_2, and multiplets at δ 2.35 and 1.60 for the α- and β-CH_2 groups, which are deshielded by the proximate carbonyl function. Similarly, all ^{13}C NMR spectra displayed the expected alkanoic ester resonances, namely, at δ 14.1, 22.7, and 32.0 for terminal CH_3 and its two adjacent CH_2 groups, at 33.9 and 24.7 for the α- and β-CH_2 groups, and at 29.4 $\pm$0.3 (several lines) for the bulk of chain-internal CH_2 groups. The ^{1}H resonances for the carbohydrate core are listed in Tables I–III, inspection of which reveals that, for a given substitution pattern, interchange of palmitoyl and stearoyl has no discernible effect on the spectrum, within the bounds of instrumental accuracy. The same is true for the carbohydrate ^{13}C resonances (Table IV). The following regularities may be noted. *O*-Acylation causes the usual deshielding of ring protons, clearly differentiating 2-*O*-acyl and 3-*O*-acyl structures by respective doublets of doublets for H-2 that occur at δ 4.85–5.0 in benzylidene derivatives (Tables II and III) and at δ 4.7 in the deprotected compounds (Table I), and by triplets for H-3 at δ 5.3–5.6 (Tables II and III) and 5.2–5.3 (Table I). Anomeric protons (H-1′) in benzylidenated but otherwise unsubstituted (or silylated) glucose units resonate near δ 5.10, provided

* The proportion of these fast-moving by-products was noticeably increased relative to **20** when a catalytic amount of 4-dimethylaminopyridine was added to the reaction medium.

** When the mixture of monostearoyl by-products, suspected of carrying more than one silyl grouping, was stearoylated and the resulting product desilylated, the same diol **14** was obtained in 66% yield.

TABLE I

^{1}H NMR data for di-*O*-acyl-α,α-trehaloses [a]

Compound	Chemical shifts (δ)										
	H-1	H-1′	H-2	H-2′	H-3	H-3′	H-4	H-4′	H-5	H-5′	Others [b]
2 [c,d]	5.09d	4.88d	4.68dd	3.27dd	5.32t			3.10t	3.88dt		3.6–3.35
3 [c,d]	5.085d	4.87d	4.67dd		5.32t				3.88m		3.8–3.2
5 [c,e,f]	5.085d	4.88d	4.68dd	3.27dd	5.31t			3.10t	3.87dt		3.6–3.35
6 [d,g]	5.22d	5.11d	4.69dd	3.62dd	4.08dd	5.24dd	3.46t			3.95m	3.85–3.65
7 [d,g]	5.22d	5.13d	4.68dd	~3.6dd	4.09dd	5.23t	3.42dd			3.94m	3.8–3.5
7 [c,d]	5.03d	4.91d	4.51dd		3.80dd	5.02t	3.26t				3.7–3.3
	Coupling constants (Hz)										
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$			
2	3.5	10.1	9.4		3.5	9.4	~10	~10			
3	3.6	10	9	9.9	3.3						
5	3.4	10.1	9.4	9.9	3.4		~10	~10			
6	3.7	10.1	~9.5	~9.5	3.7	10.1	9.0				
7 [g]	3.6	10	9	9.9	3.6	~9.5	~9.5				
7 [c]	~3.5	10.1	9	9.2	3.8	~9.5	~9.5				

[a] Data recorded after deuterium exchange. See text for resonances of the lipid-chain protons. [b] Range in which occurred overlapping multiplets for H-6a,6b,6′a,6′b and those ring protons for which δ values are not specified. [c] In $(CD_3)_2SO$. [d] At 200 MHz. [e] At 300 MHz. [f] A 200-MHz spectrum taken at room temperature without deuterium exchange showed O*H*-signals at δ 5.32 (d, O*H*-4), 5.00 (d, O*H*-4′), 4.95–4.94 (2 d, O*H*-2′,3′), 4.55 (t, O*H*-6), and 4.43 (t, O*H*-6′). At 50°C, the O*H*-4 signal moved upfield to δ 5.17 (thus revealing a clear H-3 triplet at δ 5.31), whereas the OH-2′,3′ signals moved to δ ~4.73 (thereby obscuring the downfield part of the H-2 doublet of doublets). [g] In $(CD_3)_2CO$.

TABLE II

^{1}H NMR data [a] for acylated 4,6:4′,6′-di-*O*-benzylidene-α,α-trehaloses

Compound	Chemical shifts [b] (δ)							
	PhC*H*	H-1	H-2	H-3	H-4	H-5	H-6*ax*	H-6*eq*
9	5.53	5.34	4.875	4.23	3.58	4.3–3.6		
	5.48	5.12	~3.7	3.97	3.48			
10	5.53	5.345	4.875	4.21	3.57	[c]	3.71(2 H)	4.29
	5.47	5.11	3.64	3.98	3.46	3.77		[c]
11 [d]	5.49	5.34	4.99	5.64	3.67	4.17	3.73	4.30
	5.44	5.09	~3.63	4.01	3.45	3.775	3.63	4.08
12	5.49	5.35	5.00	5.64	3.66	4.18	3.73	4.31
	5.46	5.12	~3.63	4.04	3.46	3.78	3.65	4.09
13 [e]	5.49(2 H)	5.35	5.00	5.62	3.68	4.16	3.73	4.30
		5.16	~3.68	4.07	3.49	~3.8	3.66	4.10
14 [d,f]	5.50	5.34	5.00	5.63	3.66	4.16	3.73	4.30
	5.44	5.06	~3.63	3.99	3.44	3.76	3.62	4.07
23 [d]	5.52	5.395	4.90	4.25	3.58	3.85	3.69	4.15
	5.47	5.16	3.73	5.345	3.63	3.98	3.71	4.28
24	5.52	5.385	4.89	4.25	3.57	3.85	3.68	4.14
	5.46	5.155	3.72	5.34	3.62	3.975	3.71	4.27
25	5.47	5.235	3.71	5.55	3.63	3.98	3.71	4.275
26	5.48	5.39	5.015	5.61	4.3–3.5			
	5.46	5.16		5.34				
27	5.47	5.31(2 H)	4.98	5.63	4.3–3.5			
	5.51		4.85	4.28				
28 [g]	5.49(2 H)	5.32(2 H)	4.87, 4.92	4.21, 4.24	3.55, 3.68	4.15–3.8		
29 [g]	5.46(2 H)	5.34(2 H)	5.00, 5.06	5.62(2 H)	3.66, 3.70	4.15–3.8		
30	5.48	5.39	5.02	5.62	[h]	[i]	[h]	4.30
	5.46	5.16	[h]	5.34	[h]	[h]	[h]	[i]

[a] For $CDCl_3$ solutions at 200 MHz, unless otherwise indicated. Data for unprimed and primed positions are given in upper and lower lines, respectively; data pertaining to both sugar units are given in a center line. [b] See text for resonances of the lipid-chain protons. Signal multiplicities for corresponding carbohydrate protons in different compounds were the same, and *J* values were in accord with α-D-glucopyranoside $^4C_1$ chair geometry, deviating from one another only within the limits of instrumental accuracy. Values found for **11** are representative: $J_{1,2}$ 3.9, $J_{2,3}$ 10.0, $J_{3,4}$ 9.7, $J_{4,5}$ 9.5, $J_{1',2'}$ 3.7, $J_{2',3'} \approx J_{3',4'} \approx 9.3$, and $J_{4',5'}$ 9.5 Hz. [c] Part of unresolved m (2 H) at δ ~ 4.1. [d] At 300 MHz; assignments confirmed by COSY experiment. [e] The solvent contained moisture, which caused slightly increased, anomalous shifts for H-1′, H-2′, H-3′, and one of the PhC*H* signals. See also footnote *f*. [f] In moist solvent the two PhC*H* signals coincided at δ 5.49, and increased shifts were observed for H-1′ (5.15), H-2′ (~3.69), and H-3′ (4.06). [g] The $OCOCH_2Br$ group in **28** and **29** gave 2-proton singlets at δ 3.92 and 4.09, respectively. [h] Part of unresolved m (6 H) at δ 3.85–3.55. [i] Part of unresolved m (2 H) at δ 4.15–4.0.

TABLE III

^{1}H NMR data [a] for 2′,3′-*O*-silylated derivatives of acylated 4,6:4′,6′-di-*O*-benzylidene-α,α-trehaloses

Compound	Chemical shifts [b] (δ)							
	PhC*H*	H-1	H-2	H-3	H-4	H-5	H-6*ax*	H-6*eq*
15 [c]	5.52(2 H)	5.355 5.10	4.85 3.87	4.22 4.13	3.57 3.50	[d] ~ 3.8	3.72, 3.68	4.23 [d]
16	5.54 5.45	5.18(2 H)	3.71 3.9 [e]	5.34 4.16	3.60 3.51	[d] 3.9 [e]	3.69(2 H)	4.28 [d]
17	5.51 5.46	5.35 5.11	4.95 3.89	5.64 4.19	3.64 3.49	~ 4.2, ~ 3.77	3.72, 3.67	4.32 4.11
18	5.51 5.46	5.34 5.10	4.96 3.89	5.64 4.18	3.635 3.49	~ 4.2, ~ 3.77	3.72, 3.67	4.31 4.105
19	5.51 5.46	5.34 5.10	4.965 3.885	5.64 4.18	3.64 3.49	~ 4.2, ~ 3.77	3.72, 3.67	4.32 4.11
20	5.51(2 H)	5.35 5.09	4.85 3.86	4.22 4.12	3.56 3.49	[d] ~ 3.78	3.72, 3.67	4.23 [d]
21	5.54 5.45	5.18(2 H)		5.34	3.51			
22 [c]	5.51 5.46	5.345 5.11	4.97 3.89	5.64 4.18	3.64 3.49	~ 4.2, ~ 3.8	~ 3.7(2 H)	4.33 4.11

[a] See footnote *a* of Table II for particulars. [b] All compounds displayed multiple signals near δ 1.0 due to isopropyl groups. The *J* values of carbohydrate protons corresponded closely to those exemplified in footnote *b* of Table II, except that introduction of the silyl ether ring entailed minor changes in the couplings of H-1′,2′,3′, and 4′; $J_{1',2'}$ 4 and $J_{2',3'} \approx J_{3',4'} \approx 8.7$, found for **15** and **22** are typical values. [c] At 300 MHz; assignments confirmed by COSY experiment. [d] Part of unresolved m (2 H) at δ 4.2–4.1. [e] Part of unresolved m.

the other glucose unit is 2-acylated or 2,3-diacylated (see compounds **9–15**, **17–20**, and **22**); if OH-2 is free, H-1′ is slightly deshielded (δ 5.18, see **16** and **21**). The anomeric proton (H-1) in benzylideneglucose units acylated at O-2, or at O-2 and O-3, generally resonates at δ 5.35 ±0.01 when the positions O-2′ and O-3′ in the other unit are either unsubstituted (**9–14**) or silylated (**15**, **17–20**, and **22**). Deshielding of H-1 is marginally increased when the second unit bears a 3′-acyl group (δ 5.39 for **23**, **24**, **26**, and **30**), whereas a 2′-acyl group in the second ring appears to have a small opposite effect (δ 5.31–5.32 for **27** and **28**; in **29** the two effects nearly cancel). Some inter-residue shielding dependencies in trehalose-type disaccharides have been observed before[12]. The anomeric signal for 3-acylated rings having a fee OH-2 group normally occurs at δ 5.16–5.18 (see H-1 for **16** and **21**, and H-1′ for **23**, **24**, **26**, and **30**; the symmetrical 3,3′-dipalmitate **25** is an exception, with δ 5.24 for H-1,1′). Chemical shifts of protons in acylated 2-positions fall into two groups marked by a small but distinct difference: They resonate at δ 4.87 ± 0.02 when the vicinal OH-3 group is free, and at δ 4.98 ±0.03 when it is acylated (Tables II and III). A similar, if noticeably larger, effect is observed in most 3-acylated compounds where, depending on whether OH-2 is free or acy-

TABLE IV

^{13}C NMR data [a] for α,α-trehalose derivatives

Compound	Chemical shifts [b] (δ)					
	C-1	C-2	C-3	C-4	C-5	C-6
2 [c]	90.8 94.4	70.6 71.3	72.2 72.8	67.6 69.8	72.4 73.5	60.0 60.7
5 [c,d]	90.7 94.3	70.5 71.3	72.1 72.8	67.5 69.9	72.5 73.5	59.9 60.7
9 [d]	92.4 94.8	72.8 71.9	68.2 71.0	81.1 80.6	63.1, 62.7	68.6, 68.5
10	92.4 94.8	72.8 71.8	68.4 70.8	81.1 80.5	63.1, 62.7	68.6, 68.5
11	92.3 94.8	70.8 72.1	68.6 71.1	79.2 80.8	63.2, 63.1	68.6 68.6
14 [d,e]	92.2 94.9	70.8 72.0	68.5 70.9	79.2 80.7	62.9 63.2	68.5 68.5
15	91.8 94.4	72.9 75.1	68.6 73.4	81.4 81.0	62.8, 62.3	68.8, 68.7
16 [d,e]	94.4, 94.0	71.9 75.2	72.1 73.3	78.5 81.1	62.7 62.8	68.8 68.8
17 [d]	92.0 94.5	71.0 75.3	68.6 73.4	79.3 81.1	62.8, 62.6	68.8, 68.7
18	92.0 94.5	71.0 75.2	68.6 73.4	79.3 81.0	62.8, 62.6	68.8 68.8
19	92.0 94.5	71.0 75.3	68.6 73.4	79.3 81.1	62.8, 62.6	68.7 68.7
20	91.8 94.4	72.9 75.1	68.6 73.5	81.4 81.0	62.8, 62.3	68.8, 68.7
22	92.0 94.5	71.0 75.2	68.6 73.4	79.3 81.1	62.8, 62.5	68.7, 68.6
23 [d,e]	92.2 94.9	72.7 71.6	68.4 72.2	81.1 78.2	63.4, 63.0	68.6, 68.5
24	92.3 94.9	72.8 71.5	68.3 72.3	81.2 78.3	63.4, 63.1	68.5, 68.4
25	93.8	72.0	71.5	79.0	63.4	68.9
28 [f]	92.4, 92.15	74.1(2 C), 72.6(2 C)		81.0, 80.8	63.0, 62.9	68.4(2 C)

[a] For $CDCl_3$ solutions at 50.3 MHz, unless noted otherwise. Data for unprimed and primed positions are given in upper and lower lines, respectively; data not attributable to a particular glucose unit are given in a center line. [b] In addition to the lipid-chain carbon signals mentioned in the text, all compounds showed ester CO signals (δ 174.5–172.5). Benzylidene derivatives gave 4 pairs of closely-spaced (or sometimes coinciding) signals for the two phenyl groups near δ 137, 129, 128, and 126, and two signals for PhC at δ 102.0 $\pm$0.3 and 101.4 $\pm$0.4 (except for symmetrical **25**, which gave only one set of signals). Silylated compounds additionally showed multiple signals for Me ($\delta \sim 17.0$) and $SiCHMe_2$ ($\delta \sim 12.0$) of the isopropyl groups. [c] In $(CD_3)_2SO$. [d] At 75.4 MHz. [e] Assignments verified by HETCOR experiment. [f] Signals for $COCH_2Br$ occurred at δ 167.1 and 24.5.

lated, H-3 resonates at δ 5.34 or 5.61–5.64. Again, the symmetrical 3,3′-dipalmitate **25** was an exception, showing δ 5.55 for H-3,3′.

Antigenic properties.—A large number of synthetic glycolipids comprising cord factor analogs and "mirror" pseudo cord factors, i.e., trehalose 6,6′-diesters as well as trehalose derivatives having lipid chains attached to functionally modified terminal positions, were recently screened as potential immunoreactants for the serodiagnosis of tuberculosis by the enzyme-linked immunosorbent assay (ELISA), and several of the synthetic compounds examined were found to be effective antigens reacting with sera from tuberculosis patients[13]. In continuation of these studies the new trehalose 2,3- and 2,3′-diesters are currently being evaluated in the same way, and preliminary results obtained for some of them indicate high levels of immunoreactivity, up to 75% of that of the natural, structurally analogous antigen SL-IV[14]. It therefore appears that antigenicity is basically afforded by normal C_{16} and C_{18} ester chains, although it is enhanced when a certain proportion of longer and more-complex chains are present, as is the case[6] for SL-IV.

EXPERIMENTAL

General methods.—Solvents used in stannylenations and acylations were rigorously dried by standard procedures, and the reactions were performed under careful exclusion of atmospheric moisture. Anhydrous Na_2SO_4 was used for drying solutions of products in nonpolar solvents. Thin-layer chromatography (TLC) was performed with precoated silica gel plates, and the spots were revealed on a hot-plate after spraying with 5% H_2SO_4 in EtOH. Chromatographic columns contained Merck silica gel, 70–230 or 230–400 mesh as appropriate, or equivalent material. The following chromatographic solvent combinations (v/v) were used unless stated otherwise: EtOAc–hexanes, (*A*) 1:1, (*B*) 1:3, (*C*) 1:4, (*D* 1:10, and (*E*) 1:20; and (*F*) 50:60:2.5:3 $CHCl_3$–acetone–MeOH–water. Melting points were taken in capillaries in a Gallenkamp electrothermal apparatus. Optical rotations were measured in a Perkin–Elmer 241 polarimeter at room temperature, in $CHCl_3$ solutions unless otherwise specified.

4,6:4′,6′-Di-O-benzylidene-2-O-palmitoyl-α,α-trehalose (**9**).—A suspension of diacetal[7] **8** (520 mg, 1 mmol) and Bu_2SnO (284 mg, 1.1 mmol) in dry benzene (300 mL) was boiled under reflux for 2 days, with azeotropic removal of water in a Dean–Stark trap filled with 4A molecular sieves. The resulting, almost clear solution was cooled to 25°C, palmitoyl chloride (0.37 mL, 1.2 mmol) and Et_3N (1 mL) were added, and the mixture was heated overnight at 50–60°C. The solvent was removed, and a solution of the residue in CH_2Cl_2 was washed with water, dried, and evaporated. The main product **9** (R_f 0.3, TLC with solvent *A*) was separated from fast-moving by-products by column chromatography using sequentially solvents *D*, *B*, and *A* as eluents; yield, 0.50 g (66%); mp 138–141°C (from MeOH); for NMR data see Tables II and IV. Anal. Calcd for $C_{42}H_{60}O_{12} \cdot 0.5H_2O$ (765.9): C, 65.86; H, 8.03. Found: C, 65.63; H, 7.78.

For further characterization a sample of **9** was acetylated (Ac_2O, pyridine; 25°C) to give, after conventional processing, a syrupy *triacetate*; ^1H NMR (200 MHz, $CDCl_3$) data: δ 7.4–7.3 (m, Ph), 5.60 (t, splittings 9.8 Hz, H-3,3′), 5.47 (d, splitting 4.0 Hz, H-1,1′), 4.98 (dd, splittings 4 and 10 Hz, H-2,2′), 4.13 (m, H-5,5′), 4.0–3.9 (m, H-6*eq*, 6′*eq*), 3.8-3.6 (m, H-4,4′, 6*ax*, 6′*ax*), 2.4–2.25 (m, α-CH_2 of Pa), 2.11, 2.05, and 2.03 (3 s, 3 OAc), ~ 1.57 (m, β-CH_2 of Pa), 1.23-1.17 (bulk of CH_2 in Pa), and 0.85 (t, CH_3 of Pa).

4,6:4′,6′-Di-O-benzylidene-2,3′-di-O-palmitoyl-α,α-trehalose (**24**) *and its 3,3′-di-O-palmitoyl isomer* **25**.—Compound **8** (520 mg, 1 mmol) was treated with Bu_2SnO (270 mg) as just described, but in refluxing toluene during 8 h, after which the solvent was evaporated and the residue dried in a high vacuum. The material was dissolved in 1,4-dioxane (10 mL), palmitoyl chloride (0.62 mL, 2 mmol), Bu_4NI (250 mg), and 4A molecular sieves (5 g) were added, and the mixture was efficiently stirred for 3 days at room temperature. The molecular sieves were filtered off and washed well with EtOAc, and the combined filtrate and washings were concentrated for column chromatography. Elution was started with hexane and continued with 1:15, 1:10, 1:5, and 1:1 EtOAc–hexane mixtures, to give a fraction (30 mg) of fast-moving products (presumably triesters, R_f 0.7 in TLC with solvent *B*), a mixed fraction (~ 20 mg, R_f 0.7–0.6), a fraction containing chiefly **25** (20 mg, R_f 0.6), and fractions of pure **24** (R_f 0.5) amounting to 350 mg (35%). Final eluates contained small amounts of starting **8** and monopalmitate **9** (^1H NMR).

Compound **24** had: mp 148–150°C; $[\alpha]_D$ +48° (*c* 0.1); see Tables II and IV for NMR data. Anal. Calcd for $C_{58}H_{90}O_{13} \cdot H_2O$ (1013.3): C, 68.74; H, 9.15. Found: C, 68.59; H, 8.70

Compound **25** was characterized by NMR spectra only (Tables II and IV).

4,6:4′,6′-Di-O-benzylidene-2-O-stearoyl-α,α-trehalose (**10**), *4,6:4′,6′-di-O-benzylidene-2,3′-di-O-stearoyl-α,α-trehalose* (**23**), *and the related tristearate mixture* (**26** + **27**).—A suspension of **8** (520 mg, 1 mmol) and Bu_2SnO (260 mg) in toluene (250 mL) was boiled overnight under reflux, with azeotropic removal of water by a Dean–Stark trap. 1,4-Dioxane (100 mL) was added and refluxing continued for 2 h whereby the solution became clear. After the portionwise addition of stearoyl chloride (0.74 g, 2.4 mmol) at 25°C, the mixture was heated for 2 h at 50–60°C and then processed and subjected to column chromatography as described for **9**. There was obtained a fast-moving fraction (R_f 0.9) of triesters **26** + **27** (55 mg, 4%), a fraction (R_f 0.7) of distearate **23** (260 mg, 25%), slow-moving (R_f 0.1) monostearate **10** (414 mg, 51%), and some unreacted **8** (70 mg, 13%; R_f 0.0 [TLC with solvent *B*]).

Compound **10** showed mp 136–138°C; $[\alpha]_D$ +78.3° (*c* 0.5); see Tables II and IV for NMR data. Anal. Calcd for $C_{44}H_{64}O_{12} \cdot 1.5H_2O$ (812.0): C, 65.08; H, 8.32. Found: C, 64.83; H, 8.18.

A sample of **10** was acetylated as for **9**, to give a syrupy triacetate whose ^1H NMR spectrum (200 MHz, $CDCl_3$) was virtually identical with that of acetylated **9**.

Compound **23** has mp 136–138°C; $[\alpha]_D$ +63.5° (*c* 0.1); see Tables II and IV for NMR data. Anal. Calcd for $C_{62}H_{98}O_{13}$ (1051.5): C, 70.82; H, 9.40. Found: C, 71.00; H, 9.33.

The mixture **26** + **27** had mp 95–98°C; $[\alpha]_D$ +49.6° (*c* 0.5); see Table II for ^1H NMR data. Anal. Calcd for $C_{80}H_{132}O_{14}$ (1317.9): C, 72.91; H, 10.10. Found: C, 72.94; H, 9.96.

4,6: 4',6'-Di-O-benzylidene-2-O-palmitoyl-2',3'-O-(tetraisopropyldisiloxane-1,3-diyl)-α,α-trehalose (**15**) *and its 3-O-palmitoyl isomer* (**16**).—To an ice-cooled solution of **9** (760 mg, 1 mmol) in pyridine (5 mL) was added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (0.39 mL, 1.2 mmol). After standing for 2 days at room temperature the mixture was poured into ice–water and the product extracted with EtOAc. The extract was successively washed with water, M HCl, aq $NaHCO_3$, and water, dried, and concentrated. Column chromatography of the crude product, employing hexane followed by solvent *D* as eluents, gave a minor proportion of a fast-moving by product (R_f 0.7; not further investigated), a fraction (30 mg, 3%) of 3-palmitate **16** (R_f 0.6), and the main product **15** (400 mg, 40%; R_f 0.55); traces of less-mobile by-products present were not examined (TLC with solvent *B*).

Compound **15** was a syrup; $[\alpha]_D$ +45° (*c* 0.1); see Tables III and IV for NMR data. Anal. Calcd for $C_{54}H_{86}O_{13}Si_2$ (999.4): C, 64.89; H, 8.67. Found: C, 64.69; H, 8.78.

Compound **16** was a syrup; $[\alpha]_D$ +52° (*c* 0.5); see Tables III and IV for NMR data. Anal. Calcd for $C_{54}H_{86}O_{13}Si_2 \cdot 0.5H_2O$ (1008.4): C, 64.31; H, 8.69. Found: C, 64.08; H, 8.47.

4,6: 4',6'-Di-O-benzylidene-2,3-di-O-palmitoyl-2',3'-O-(tetraisopropyldisiloxane-1,3-diyl)-α,α-trehalose (**17**).—Compound **15** (165 mg, 0.165 mmol, of a chromatographic fraction containing some **16**) in pyridine (5 mL) was treated overnight at 50°C with palmitoyl chloride (0.075 mL, 0.25 mmol) and 4-dimethylaminopyridine (20 mg). The mixture was poured into ice–water, and the oily precipitate was taken up in hexane, which also served to extract the supernatant several times. The extract was washed with water, dried, concentrated, and subjected to flash chromatography (solvent *D*) to furnish syrupy **17** (170 mg, 83%); R_f 0.8 (TLC with solvent *A*); $[\alpha]_D$ +35° (*c* 0.3); see Tables III and IV for NMR data. Anal. Calcd for $C_{70}H_{116}O_{14}Si_2$ (1237.8): C, 67.92; H, 9.45. Found: C, 68.09; H, 9.33.

4,6:4',6'-Di-O-benzylidene-2-O-palmitoyl-3-O-stearoyl-2',3'-O-(tetraisopropyldisiloxane-1,3-diyl)-α,α-trehalose (**18**).—Chromatographically pure **15** (150 mg) was acylated as just described for the preparation of **17**, but with stearoyl chloride (0.076 mL), to give syrupy **18** (161 mg, 81%); $[\alpha]_D$ +35° (*c* 0.3); see Tables III and IV for NMR data.

4,6: 4',6'-Di-O-benzylidene-3-O-palmitoyl-2-O-stearoyl-2',3'-O-(tetraisopropyldisiloxane-1,3-diyl)-α,α-trehalose (**19**).—Chromatographically homogeneous **16** (50 mg) was acylated as described for the preparation of **17**, but with stearoyl chloride (0.025 mL), to give syrupy **19** (55 mg, 87%); $[\alpha]_D$ +34° (*c* 1.3); see Tables

III and IV for NMR data. Anal. Calcd for $C_{72}H_{120}O_{14}Si_2$ (1265.9): C, 68.31; H, 9.56. Found: C, 68.12; H, 9.38.

4,6: 4',6'-Di-O-benzylidene-2-O-stearoyl-2',3'-O-(tetraisopropyldisiloxane-1,3-diyl)-α,α-trehalose (**20**) *and its 3-O-stearoyl isomer* **21**.—To a cooled (0°C) solution of **10** (270 mg, 0.34 mmol) in dry pyridine (5 mL) was added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (0.13 mL, 0.4 mmol). No reaction was observed during 16 h at room temperatere, but it commenced on heating at 50–60°C (TLC with solvent *C*). After 10 h, another 0.06 mL of silylating agent was added, and heating was continued for 16 h after which TLC showed a major spot at R_f 0.7 (**20**) accompanied by a minor spot at R_f 0.75 (**21**), and 2 spots (R_f 0.85–0.82) for less-polar, as well as 2 spots (R_f 0.63 and 0.50) for more-polar by-products. Procesing of the reaction mixture as described for **15** and **16**, and column chromatography of the crude product (eluents: hexane followed by solvents *D* and *B*) gave fractions containing the two nonpolar by-products (R_f 0.85–0.82), which were not separated (70 mg, ~ 16%), followed by mixed fractions (120 mg, 34%) of **20** and **21**, and a fraction of pure **20** (110 mg, 31%), all syrupy. Further elution of the column gave trace amounts of unidentified materials (R_f 0.63–0.5), and unchanged, crystalline **10** (30 mg, 11%).

The 2-stearate **20** had $[\alpha]_D$ +47° (*c* 1); see Tables III and IV for NMR data. Anal. Calcd for $C_{56}H_{90}O_{13}Si_2$ (1027.5): C, 65.46; H, 8.83. Found: C, 65.33; H, 8.77.

The mixture of **20** and **21** gave all the ^1H NMR signals of **20** and additional signals attributable to **21**; see Table III. The mixture of nonpolar by-products showed NMR signals for carbohydrate protons and ^{13}C atoms very similar to those given by **20**, but it exhibited signals for more than four isopropyl groups (see Discussion).

4,6: 4',6'-Di-O-benzylidene-2,3-di-O-stearoyl-2',3'-O-(tetraisopropyldisiloxane-1,3-diyl)-α,α-trehalose (**22**).—A mixture of **20** and **21** (120 mg, ~ 0.12 mmol) was treated with stearoyl chloride (45 mg, 0.15 mmol) in pyridine (2 mL) containing 4-dimethylaminopyridine (20 mg), as described for the palmitoylation of **15**. Processing and purification of the product, performed as for **17**, gave syrupy **22** (140 mg, 92%); $[\alpha]_D$ +35.4° (*c* 0.4); see Tables III and IV for NMR data. Anal. Calcd for $C_{74}H_{124}O_{14}Si_2$ (1293.9): C, 68.69; H, 9.66. Found: C, 68.79; H, 9.90.

4,6: 4',6'-Di-O-benzylidene-2,3-di-O-palmitoyl-α,α-trehalose (**11**).—A M solution of Bu_4NF in oxolane (0.4 mL) was added to an ice-cooled solution of **17** (56 mg) in water-saturated EtOAc (2 mL), and the mixture was stirred for 3 h at room temperature, then diluted with EtOAc, washed with water, dried, and concentrated by evaporation. The crude product, which showed a single spot (R_f 0.15) in TLC with solvent *A*, was passed through an SiO_2 column by elution with solvent *B* followed by solvent *A* to give crystalline **11** (40 mg, 89%); mp 116–117°C; $[\alpha]_D$ +65° (*c* 0.1); see Tables II and IV for NMR data. Anal. Calcd for $C_{58}H_{90}O_{13}$ (995.3): C, 69.99; H, 9.11. Found: C, 69.88; H, 8.88.

4,6: 4',6'-Di-O-benzylidene-2-O-palmitoyl-3-O-stearoyl-α,α-trehalose (**12**).—Performed as for **17**, the desilylation of **18** (40 mg) yielded crystalline **12** (24 mg, 74%);

mp 118.5–120°C; $[\alpha]_D$ +45° (*c* 1.2); see Tables II and IV for NMR data. Anal. Calcd for $C_{60}H_{94}O_{13}$ (1023.4): C, 70.42; H, 9.26. Found: C, 70.30; H, 9.10.

4,6:4',6'-Di-O-benzylidene-3-O-palmitoyl-2-O-stearoyl-α,α-trehalose (**13**).— Performed as for **17**, the desilylation of **19** (40 mg) yielded crystalline **13** (20 mg, 62%); mp 112–113°C; $[\alpha]_D$ +51° (*c* 0.1); see Tables II and IV for NMR data. Anal. Calcd for $C_{60}H_{94}O_{13}$ (1023.4): C, 70.42; H, 9.26. Found: C, 70.38; H, 9.27.

4,6:4',6'-Di-O-benzylidene-2,3-di-O-stearoyl-α,α-trehalose (**14**).—Performed as for **17**, the desilylation of pure **22** (100 mg) furnished crystalline **14** (70 mg, 86%); mp 110–113°C; $[\alpha]_D$ +48° (*c* 0.5); see Tables II and IV for NMR data. Anal. Calcd for $C_{62}H_{98}O_{13}$ (1051.4): C, 70.82; H, 9.40. Found: C, 70.65; H, 9.25.

Compound **14** was also obtained (yield, 66%) when the mixture of nonpolar by-products (R_f 0.85–0.82) isolated chromatographically from crude **20** was stearoylated (compare the preparation of **22**) and the resulting product mixture was directly desilylated.

2,3-Di-O-palmitoyl-α,α-trehalose (**2**).—A suspension of **11** (40 mg) in a solution of I_2 (20 mg) in MeOH (2 mL) was boiled under reflux for 2 h, after which the conversion of **11** (R_f 0.15) into **2** (R_f 0.05) was complete (TLC with solvent *A*). The solvent was removed in a rotary evaporator without warming, and I_2 was extracted from the solid residue with CH_2Cl_2. The crude material was passed through a short SiO_2 column by means of EtOAc to give crystalline **2** (30 mg, 89%); mp 140–141°C; R_f 0.2 (TLC, solvent *F*); see Tables I and IV for NMR data. Anal. Calcd for $C_{44}H_{82}O_{13} \cdot H_2O$ (837.1): C, 63.13; H, 10.11. Found: C, 63.12; H, 9.73.

2-O-Palmitoyl-3-O-stearoyl-α,α-trehalose (**3**).—Compound **12** (20 mg) was debenzylidenated as just described for **11**, to give **3** as an amorphous solid; mp 138–145°C; see Table I for ^1H NMR data. Anal. Calcd for $C_{46}H_{86}O_{13}$ (847.1): C, 65.21; H, 10.23. Found: C, 65.20; H, 10.26.

2,3-Di-O-stearoyl-α,α-trehalose (**5**).—Compound **14** (100 mg) was deacetalated by the procedure detailed for **11**, giving crystalline **5** (68 mg, 82%); mp 141–144°C; see Tables I and IV for NMR data.

2,3'-Di-O-stearoyl-α,α-trehalose (**6**).—Deacetalation of **23** (200 mg) by the foregoing procedure afforded crystalline **6** (130 mg, 78%); mp 147–148°C; see Table I for ^1H NMR data.

2,3'-Di-O-palmitoyl-α,α-trehalose (**7**).—Compound **24** (80 mg) was treated with 90% CF_3CO_2H (2 mL) for 2 h at room temperature. The acid was evaporated at low temperature under reduced pressure, and a solution of the residue in CH_2Cl_2 was washed with water, dried, and evaporated. The crude product was purified by column chromatography with EtOAc as the eluent, which provided crystalline **7** (29 mg, 45%); mp 139–140°C; see Table I for ^1H NMR data. Anal. Calcd for $C_{44}H_{82}O_{13}$ (819.1): C, 64.52; H, 10.08. Found: C, 64.33; H, 9.89.

4,6:4',6'-Di-O-benzylidene-2'-O-bromoacetyl-2-O-palmitoyl-α,α-trehalose (**28**).— Benzene, 1,4-dioxane, and MeOH were dried over P_2O_5, Na, and Mg, respectively. A solution of 2-palmitate **9** (112 mg) in 10:1 benzene–MeOH (8 mL) and Bu_2SnO

(37 mg) were heated for 45 min at the reflux temperature and then overnight at 65°C. The solvent was evaporated, and the residual oil (R_f 0.4, TLC with solvent *A*) was dried in vacuo over P_2O_5, dissolved in 1,4-dioxane (3 mL) and, after the addition of Et_3N (1 drop), treated with bromoacetyl bromide (3 × 0.01 mL, added in the course of 45 min). Conversion of the stannylenated compound (R_f 0.4) into a major product (R_f 0.8) and several trace products was indicated by TLC (solvent *A*). Concentration of the mixture and column chromatography (4 g of SiO_2; solvent *B*) afforded pure **28** (104 mg, 80%); see Tables II and IV for NMR data.

4,6 : 4',6'-Di-O-benzylidene-2'-O-bromoacetyl-2-O-palmitoyl-3,3'-di-O-stearoyl-α,α-trehalose (**29**).—A solution of **28** (96 mg) and stearoyl chloride (95 mg) in dry 1,2-dichloroethane (3.5 mL) was kept at room temperature for 1 h, after which TLC (solvent *B*) indicated nearly complete replacement of **28** (R_f 0.3) by one major product (R_f 0.8) and traces of several by-products. Concentration of the solution and chromatography of the product on SiO_2 (10 g), using solvent *E* followed by solvent *D* as eluents, furnished homogeneous **29** (88 mg, 57%), R_f 0.8 (TLC, solvent *B*); for NMR data, see Tables II and IV.

4,6 : 4',6'-Di-O-benzylidene-2-O-palmitoyl-3,3'-di-O-stearoyl-α,α-trehalose (**30**).—A solution of **29** (47 mg) and thiourea (7 mg) in 1,4-dioxane (3 mL) and 99% EtOH (3 mL) was stirred with added $NaHCO_3$ (7 mg) for 3 h at 65°C. The TLC spot for **29** (R_F 0.8) was replaced by a strong spot for **30** (R_f 0.7) and several slower trace spots (solvent *B*). Solvent removal and chromatography of the residue on SiO_2 (5 g) with solvent *D* have homogeneous **30** (32 mg, 74%) as a white solid; see Table II for 1H NMR data.

A sample of **30** was acetylated (Ac_2O–pyridine, 25°C, 16 h) to give, after conventional processing, the corresponding 2'-monoacetate. Its 200-MHz ^1H NMR spectrum ($CDCl_3$) was indistinguishable from those of the triacetates of **9** and **10** in the carbohydrate proton region and differed from them only in showing a single OAc signal (δ 2.10, 3H) and threefold intensities of the lipid proton signals.

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REFERENCES

1 (a) C. Asselineau and J. Asselineau, *Prog. Chem. Fats Other Lipids*, 16 (1978) 59–99; (b) M.B. Goren, *Am. Rev. Respir. Dis.*, 125 (Suppl.) (1982) 50–69; (c) M.B. Goren, in G.P. Kubica and L.G.

Wayne (Eds.), *The Mycobacteria. A Source Book, Part A*, Marcel Dekker, New York, 1984, pp 379–415; (d) M.B. Goren, in M. Kates (Ed.), *Handbook of Lipid Chemistry*, Plenum Press, New York, Vol. 6, 1990, pp 363–461.

2 D.E. Minnikin, G. Dobson, D. Sesardic, and M. Ridell, *J. Gen. Microbiol.*, 131 (1985) 1369–1374.

3 (a) F. Papa, A. Laszlo, and H.L. David, *Ann. Inst. Pasteur / Microbiol.*, 139 (1988) 535–545; (b) F. Papa, A. Laszlo, H.L. David, and M. Daffé, *Proc. 4th Int. Colloq. Mycobacteria*, in *Acta Leprol.*, 7 (Suppl. 1) (1989) 98–101; (c) M. Daffé, F. Papa, A. Laszlo, and H.L. David, *J. Gen. Microbiol.*, 135 (1989) 2759–2766.

4 F. Papa, P. Cruaud, and H.L. David, *Res. Microbiol.*, 140 (1989) 569–578; P. Cruaud, C. Berlie, J. Torgal Garcia, F. Papa, and H.L. David, *Zentralbl. Bakteriol.*, 271 (1989) 481–485; P. Cruaud, J.T. Yamashita, N. Martin Casabona, F. Papa, and H.L. David, *Res. Microbiol.*, 141 (1990) 679–694.

5 M.B. Goren, O. Brokl, P. Roller, H.M. Fales, and B.C. Das, *Biochemistry*, 15 (1976) 2728–2735; M.B. Goren, B.C. Das, and O. Brokl, *Nouveau J. Chim.*, 2 (1978) 379–384.

6 A. Lemassu, M. Lanéelle, and M. Daffé, *FEMS Microbiol. Lett.*, 78 (1991) 171–176; H.H. Baer, *Carbohydr. Res.*, in press.

7 H.H. Baer and B. Radatus, *Carbohydr. Res.*, 128 (1984) 165–174.

8 S. David and S. Hanessian, *Tetrahedron*, 41 (1985) 643–663.

9 Y. Ichikawa, A. Manaka, and H. Kuzuhara, *Carbohydr. Res.*, 138 (1985) 55–64.

10 W.A. Szarek, A. Zamojski, K.N. Tiwari, and E.R. Ison, *Tetrahedron Lett.*, 27 (1986) 3827–3830.

11 M. Masaki, T. Kitahara, H. Kurita, and M. Ohta, *J. Am. Chem. Soc.*, 90 (1968) 4508–4509; A.F. Cook and D.T. Maichuk, *J. Org. Chem.*, 35 (1970) 1940–1943.

12 H.H. Baer and B. Radatus, *Carbohydr. Res.*, 157 (1986) 65–81.

13 A. Laszlo, H.H. Baer, M.B. Goren, V. Handzel, L. Barrera, and I.N. de Kantor, *Res. Microbiol.*, 143 (1992) 217–223; A. Laszlo, H.H. Baer, M.B. Goren, V. Handzel, and F. Papa, unpublished results.

14 A. Laszlo, personal communication.