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An improved synthesis of trehalose 6-mono- and 6,6′-di-corynomycolates and related esters*

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ABSTRACT

A simplified synthesis of 6-mono- and 6,6′-di-corynomycolate esters of α,α-trehalose, and related compounds, was achieved by coupling the (hydroxyl-protected) acids to the partially trimethylsilylated sugar in the presence of dicyclohexylcarbodiimide and 4-dimethylaminopyridine. As acid reactants, (2-*RS*,3-*RS*)-3-hydroxy-2-tetradecyloctadecanoic acid (DL-corynomycolic acid) and its 2*RS*,3*SR* diastereomer were prepared from methyl palmitate by sequential Claisen condensation, reduction, chromatographic separation, and saponification. Reaction with *tert*-butylchlorodimethylsilane (imidazole) gave the disubstituted ether-esters, which were converted into the required 3-*tert*-butyldimethylsilyl ethers by partial hydrolysis. 6-Linked monocorynomycolate was obtained in excellent yield (78%) from the reaction of the *RS*,*SR* acid with the known heptakis-*O*-(trimethylsilyl)trehalose, and in good yield from equimolar portions of *RS*,*RS* acid and hexakis-*O*-(trimethylsilyl)trehalose. An excess (2.5-molar portions) of the *RS*,*RS* acid gave the 6,6′-diester (69%). The mono- and di-palmitate were similarly obtained from $(Me_3Si)_6$-trehalose. The mono (*RS*,*RS*)-$(Me_3Si)_6$-trehalose coupling product was partially resolved on a silica gel column into its *RR* and *SS* diastereomers, the former corresponding to the naturally occurring trehalose monocorynomycolate. All coupling products were deprotected to free trehalose esters by treatment first with K_2CO_3 in methanol, then tetrabutylammonium fluoride–trifluoroacetic acid in oxolane.

INTRODUCTION

Mycobacteriae, Nocardiae, Rhodococci, and Corynebacteriae (order *Actinomycetales*) contain species-specific, long-chain, 2-alkyl branched, 3-hydroxy fatty acids called mycolic acids[1]. In the bacteria, these acids are mainly esterified to trehalose, to glycerol, and to cell-wall polymer[2].

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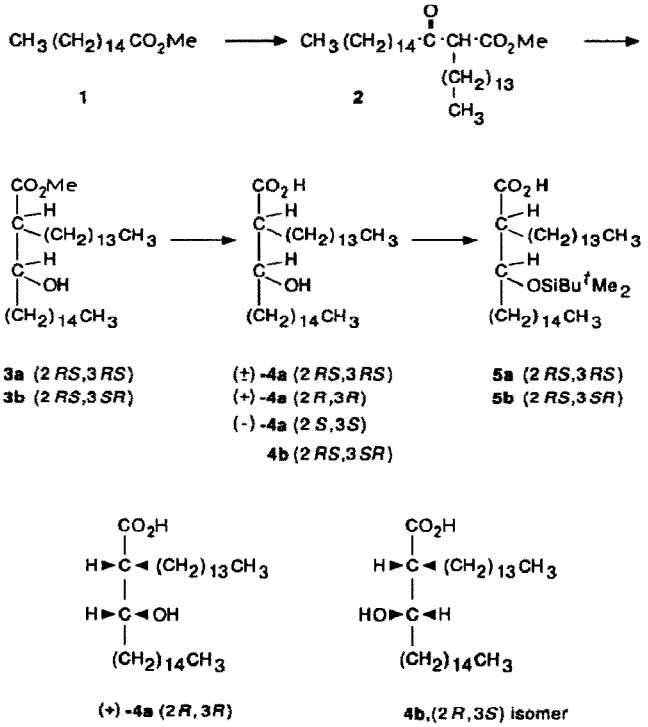
Our present interest in the biosynthesis and utilization of the mycolic acids has led us to undertake studies of the enzymes involved, including the recently discovered trehalose:mycoloyltransferase[3]. This enzyme appears to have a central role in the processing of mycolic acids. Trehalose esters of corynomycolic acid, the simplest of the mycolic acids, are convenient substrates, and thus a supply of these compounds is needed. Corynomycolic acid[4] and its trehalose esters can be isolated from the lipids of *C. diphtheriae*, but at the cost of considerable effort. The diester[5] and the monoester[6], in particular, are obtained in meager amounts. This also applies to the longer-chain trehalose mycolates of mycobacteria[7]. Thus, synthesis is an attractive alternative.

Synthetic routes to the trehalose mycolates were investigated by several groups, and procedures giving either 6,6′-diester[8,9] or 6-monoester were devised[10,11]. In most procedures, the ester bond is generated by the displacement, by mycolate ion, of a leaving group (*e.g.*, sulfonate or halide) from the C-6 atom(s) of trehalose, usually protected at its secondary positions by *O*-(trimethylsilyl) or *O*-benzyl groups. Satisfactory yields can be obtained in the esterification step, but the preparation of the derivatized intermediates tends to be tedious[8,12]. Means of reducing this preliminary effort were therefore sought and achieved by Jenkins and Goren[12], who employed the Mitsunobu reaction[13] for the condensation of 3-*O*-protected mycolic acids and unsubstituted trehalose. The method gives good yields of dimycolates, but it does not appear well suited to the preparation of monoesters. In view of this, we were led to investigate the carboxyl activation and coupling of a 3-*O*-protected corynomycolic acid to the easily obtained heptakis- or hexakis-*O*-(trimethylsilyl) derivatives of trehalose as a route to the monoester. Our results, presented herein, reveal some unanticipated advantages of this approach.

RESULTS AND DISCUSSION

For the synthesis of C_{32}-corynomycolic acid, we turned to the procedures of Lederer and coworkers[14,15]. Methyl palmitate (**1**) was subjected to self-condensation (Claisen), and the resulting keto ester **2** was reduced (sodium borohydride) to a mixture (proportions ~2:3) of the hydroxyesters **3a** and **3b**. After separation by column chromatography, the esters were saponified to yield racemic corynomycolic acid (**4a**) and its diastereomer (**4b**, also racemic), respectively.

For the protection of the 3-hydroxy function, necessary to prevent self-acylation during the coupling step, it seemed desirable to use a group that would be removed under the same conditions as trehalose-bound *O*-(trimethylsilyl) groups. We therefore tested *O*-(trimethylsilyl)- and *O*-tetrahydropyranyl-[12]corynomycolic acids, but found them unsatisfactory. The former is unstable, and the latter is a diastereomeric mixture, which makes for difficulty in purifying its acylation products. However, the 3-*O*-(*tert*-butyldimethylsilyl) derivative[16] showed a satisfactory balance between stability and ease of removal. The reaction of **4a** with *tert*-butylchlorodimethylsilane initially gave a mixture of the 3-silyl ether, the silyl ether-silyl ester, and the silyl ester, but a brief treatment with potassium carbonate cleaved the silyl ester groups, and chromatography

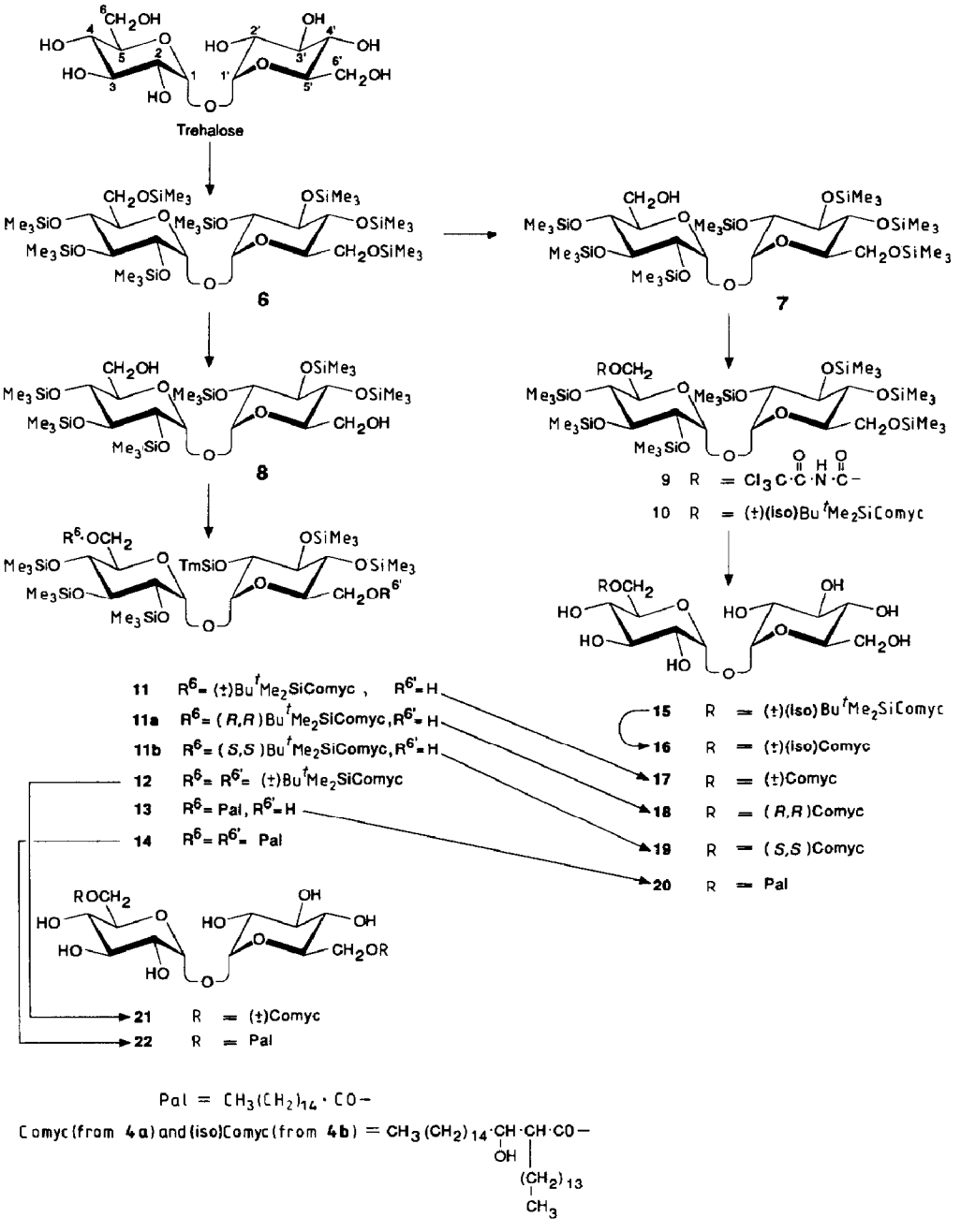


gave the desired silyl ether **5a**, plus regenerated mycolic acid that could be recycled. Similar results were obtained with the diastereomer **4b**.

Partially protected trehalose derivatives were obtained from the known 2,3,4,6,2′,3′,4′,6′-octakis-*O*-(trimethylsilyl)trehalose[17] by controlled alkaline hydrolysis using literature procedures. These gave, as desired, 2,3,4,2′,3′,4′,6′-heptakis-[18,*] (**7**) and 2,3,4,2′,3′,4′-hexakis-*O*-(trimethylsilyl)trehalose[17] (**8**), the latter in excellent yield[†]. Consistent with the symmetry of the octakis and hexakis ethers, their ^{13}C-n.m.r. spectra (excluding upfield signals for CH_3Si) consisted of six lines each. The heptakis derivative **7**, on the other hand, gave a spectrum of eight lines, including separate signals for C-1 and C-1′, and C-6 and C-6′. The ^{1}H-spectrum of **7** was also recorded, and then the compound was treated in the n.m.r. tube with trichloroacetyl isocyanate[20] to convert it into the urethane **9**. This product, not isolated, served as a model for the n.m.r.-spectroscopic characterization of the esters prepared from **7** and **8**. The salient feature of the ^{1}H-spectrum of **9** was a set of signals in the range of δ 4.0–4.6, downshifted as a result of acylation and assignable on the basis of decoupling experiments to H-6a, H-6b, and H-5.

* Primed locants are assigned to the fully substituted D-glucose unit to facilitate correlation with subsequent products.

† Heptakis- and hexakis-*O*-(trimethylsilyl)trehalose have also been prepared successfully by partial acid hydrolysis[19].



The esterification of **7** and **8** was accomplished by coupling them with the protected acids by use of dicyclohexylcarbodiimide (DCC) as the activating agent and 4-dimethylaminopyridine as catalyst[21,22]. Reaction mixtures, initially cooled, were allowed to stand for a few hours at room temperature (sufficient for palmitic acid), and

then if necessary heated to drive the acylation to completion. Preliminary experiments with long-chain mycolic acids showed that temperatures of 60–70°, in toluene solution, are required for these acids. Hence, these conditions were adopted as standard, even though a less rigorous treatment (dichloromethane under reflux) was adequate for protected corynomycolic acids. Using total reaction times of 10–14 h, excellent conversions were achieved with stoichiometric portions of acid and **7**.

The deprotection of the esterification products was studied with **10**, obtained by coupling **7** with acid **5b**. Heating with 80% aqueous acetic acid for 30 min under reflux gave apparent deprotection, but the mass and ^{1}H-n.m.r. spectra of the product (**15**) showed that it retained the *tert*-butyldimethylsilyl group. To effect complete desilylation, we adopted a two-stage procedure (see Experimental section) involving treatment first with methanolic potassium carbonate, and then tetrabutylammonium fluoride–trifluoroacetic acid in oxolane. The application of this procedure to **10** furnished the trehalose monoester **16** in about 80% yield after chromatographic purification.

Although the unprotected trehalose esters are soluble in chloroform ($CDCl_3$), the ^{1}H-n.m.r. signals obtained for solutions in this solvent, even after D_2O exchange, were broad and indistinct, suggestive of molecular aggregation. However, we found that satisfactory spectra could be recorded for solutions in di-($^2H_3$)methyl sulfoxide after the addition of a drop of trifluoroacetic acid[23]. This suppressed the OH signals, leaving fairly well resolved peaks for the anomeric protons ($\delta \sim 4.85$) and for some or all of the C-6 protons [H-6a (6'a) and H-6b (6'b)], downshifted by acylation, in the range δ 4.0–4.5.

The preparation of **16** was also attempted by the displacement method (details not presented). For this purpose, **7** was converted into its 6-trifluoromethanesulfonate (triflate) and this was treated with the potassium salt of **4b**. Some **16** was obtained on deprotection of the product, but despite the use of triflate as an improved leaving group, the yield was only 16% overall.

It was evident that the direct coupling of a hydroxyl-protected acid with a trehalose derivative having a single primary OH free constitutes a convenient synthesis of trehalose 6-monoesters. However, the possibility of using the 6,6'-dihydroxy derivative **8** as the protected trehalose reactant was attractive because of the relative ease of preparation of **8**, as compared* to **7**. We, thus, tested this alternative, even though simple kinetic considerations (two equally reactive sites*) lead to the prediction that the coupling of equimolar portions of RCO_2H and **8** will give the monoester in 50% yield, at most, along with 25% (based on **8**) of diester. The surprising result was that the reaction of **8** with the protected corynomycolic acid **5a** or with palmitic acid in each case gave the monoester as the preponderant product, along with minor amounts of diester and unchanged **8**. This outcome was shown several times by the t.l.c. analysis of reaction mixtures, and verified by the isolation of the monocorynomycolate (**11**) and the

* The yield of **7** is limited by the fact that it is formed from a precursor (**6**) having two equally reactive sites (O-6 and O-6') in its molecule. Further solvolysis, at O-6' to give **8**, thus readily ensues. The isolation of **7** then involves chromatographic separation from **8** and unchanged **6**.

(deprotected) monopalmitate (**20**) in yields of 60–70%. Evidently, the acylation of one OH in **8** greatly reduces the reactivity of the second, initially equivalent, primary OH.

A t.l.c. analysis, before workup, of the reaction mixture from the coupling of **8** and **5a** showed separate spots for the two diastereomeric monoester products **11a** and **11b**. Such diastereomers necessarily result when a racemic acid is coupled to a chiral alcohol. Chromatography on neutral alumina accomplished a partial separation, and afforded modest amounts (~200 mg each) of pure **11a** and **11b**, along with their mixture (**11**). Although also a diastereomeric mixture, the coupling product (**10**) from the reaction of **7** with acid **5b** showed no tendency to separate on thin-layer plates or columns. We speculate that the chromatographic separation of **11a** and **11b** depends on the presence, in these molecules, of a free hydroxy group. However, we did not further test this hypothesis.

The deprotection of **11**, **11a**, and **11b** gave the monocorynomycolates **17**, **18**, and **19**, respectively. Simultaneous desilylation and saponification of **11a** furnished the naturally occurring *RR*-(+) enantiomer of corynomycolic acid[24], and similar treatment of **11b** provided the *SS*-(−) enantiomer. It follows that **18** is the natural form of trehalose monocorynomycolate.

The reaction of **8** with excess **5a**, or palmitic acid, gave diesters **12** and **14**, respectively, as expected. The former, as is evident from its ^{1}H-n.m.r. spectrum, is a mixture of diastereomers (three possibilities). Deprotection gave the known trehalose dicorynomycolate[25] (**21**, also a diastereomeric mixture) and dipalmitate[17] **22**.

In conclusion, our results showed that the DCC–DMAP-mediated coupling of free acids to 2,3,4,2′,3′,4′-hexakis-*O*-(trimethylsilyl)-α,α-trehalose (**8**) constitutes a convenient and versatile synthesis of trehalose 6(6′)-mycolates and other 6(6′) fatty esters. The advantage of **8** as the trehalose reactant, other than its ease of preparation, is that it serves equally well as a precursor of both mono- and di-esters. The product is determined by the proportion of fatty acid and activators employed. In view of this, the use of **8** will make possible the synthesis of mixed diesters (*cf.* ref. 25), such as *O*-acetyl-*O*-mycoloyltrehalose ("MAT")[10,26], *via* a second acylation of initially formed monoesters.

EXPERIMENTAL

General methods. — Melting points were measured with a Fisher–Johns melting point apparatus and are uncorrected. Optical rotations were determined with a Perkin–Elmer Model 141 polarimeter. N.m.r. spectra were determined in the National Magnetic Resonance Facility at Madison by use of a Bruker AM-400 or Bruker AM-500 spectrometer at ambient temperature. Samples were dissolved in $CDCl_3$ or, in the case of deprotected final products, $(^2H_6)Me_2SO$ with an added drop of trifluoroacetic acid. ^{1}H-Chemical shifts, quoted in p.p.m. from the signal of Me_4Si, were measured from the internal chloroform signal at δ 7.26 for solutions in $CDCl_3$, and at δ 8.31 for solutions in $(^2H_6)Me_2SO$. ^{13}C-N.m.r. spectra (chloroform solutions) were recorded with the Bruker AM-500 instrument, operating at 125.76 MHz; shifts were measured from the signal of internal chloroform at δ 77.24. Electron-impact mass spectra were recorded on an

A.E.I. MS DS-50 instrument. Fast-atom-bombardment[27] and californium-plasma-desorption[28] mass spectrometry were performed at the Middle Atlantic Mass Spectrometry Laboratory, Dept. of Pharmacology and Molecular Science, The Johns Hopkins University School of Medicine, Baltimore, MD. Separations were accomplished by open-column chromatography on Silica Gel 60 (70–230 mesh, Merck) or on alumina (neutral, Merck). T.l.c. was performed on silica gel plates (250 μm, Merck). Elemental analyses were done by the Galbraith Laboratories, Inc., Knoxville, TN.

Acylation. — Hydroxyl-protected fatty acid, partially trimethylsilylated trehalose (**7** or **8**), dicyclohexylcarbodiimide, 4-dimethylaminopyridine (catalyst), molecular sieves 4A (if desired), and a stirring bar were placed in a small, round-bottomed flask having a stopcock attached as a side arm. Flask and contents were vacuum dried for 3–4 h on a liquid N_2-filled Dewar vessel[29], the flask was flushed with dry N_2 gas, and toluene was added at 0°. The resulting mixture was stirred for 4–6 h, with the temperature allowed to rise to 25°, then heated at 60–70° until coupling was complete (6–8 h), as judged by t.l.c. in chloroform or 17:3 hexane–diethyl ether. Solids were removed by filtration, and the filtrate was evaporated to dryness.

Deprotection of the coupling products. — In a typical example, the coupling product (300 mg) was dissolved in chloroform (30 mL), methanolic K_2CO_3 (0.5%, 2 mL) was added, and the mixture was stirred for 3–4 h at room temperature. Water (50 mL) was added, the phases were allowed to separate, and the chloroform layer was collected, dried (Na_2SO_4), and evaporated. A solution of the residue in dry oxolane (2 mL) was treated with M tetrabutylammonium fluoride in oxolane (2 mL, 9 mol. equiv. with respect to coupling product) and trifluoroacetic acid (0.2 mL, 12 mol. equiv.) for 1.5 h at room temperature. Water was then added, the product was extracted into chloroform, and this was concentrated to dryness.

*Methyl (2*RS*,3*RS*)-* (**3a**) *and (2*RS*,3*SR*)-3-hydroxy-2-tetradecyloctadecanoate* (**3b**). — Methyl palmitate (**1**) (4.27 g, 15.8 mmol) was dried (see *Acylation*) in a flask with a side arm, and then dissolved in dry xylene (10 mL). Hexane-washed NaH from 625 mg of an 80% dispersion (20 mmol) was added, and the mixture was heated for 5 h under reflux (145°). Extraction of the mixture with chloroform after neutralization with acetic acid afforded the keto ester **2** as an off-white solid (3.96 g, 98.5%). This was dissolved in 1:1 chloroform–methanol (50 mL) and dry, powdered $NaBH_4$ (300 mg) was added to the stirred solution, in increments over a period of several minutes. After 3–4 h at room temperature, t.l.c. in chloroform showed the conversion of **2** into a mixture of two products. Water (20 mL) was added, the mixture was shaken in a separatory funnel, the organic layer was collected over anhydrous Na_2SO_4, and the aqueous layer was extracted twice with chloroform. Evaporation of the pooled organic layers gave a residue of **3a** (R_F 0.42 on t.l.c. in chloroform) and **3b** (R_F in same solvent 0.56). The diastereoisomers were separated on a silica gel column using chloroform to yield **3a** (1.28 g, 32%) and **3b** (2.13 g, 54%). Crystallized from ethanol, **3a** had m.p. 58–60°; ^{1}H-n.m.r. ($CDCl_3$): δ 3.71 (s, 3 H, OCH_3), 3.65 (m, 1 H, H-3), 2.42 (m, 1 H, H-2), 1.75–1.40 (m, CH_2), 1.31 (br. s, CH_2 and terminal CH_3), and 0.88 (t, 3 H, terminal CH_3); e.i.m.s.: m/z 492 (M − 18), 299 (M − $C_{15}H_{31}$), and 270 (methyl palmitate); lit.[15] m.p. 57–59°.

Upon crystallization from ethanol, **3b** had m.p. 70°; lit.[15] m.p. 70°. The ^{1}H-n.m.r. ($CDCl_3$) and e.i.m.s. were similar to those of **3a**.

*(2*RS,*3*RS*)-3-Hydroxy-2-tetradecyloctadecanoic acid* [(±)**4a**]. — Compound **3a** (1.2 g, 2.3 mmol) was saponified in refluxing 5% KOH in biphasic 1:1 butanol–water (40 mL, 6 h, bath temp. ~120°), and then the mixture was acidified with HCl and extracted with chloroform. Evaporation of the organic layer afforded **4a** (1.1 g, 94%), R_F 0.34 in 19:1 chloroform–methanol and 0.29 in 200:200:1 diethyl ether–hexane–acetic acid, comigrating with corynomycolic acid from *C. diphtheriae*. After crystallization from ethanol, **4a** melted at 69–70°; lit.[15] m.p. 68–69° for the synthetic, racemic compound.

Anal. Calc. for $C_{32}H_{64}O_3$ (496.86): C, 77.36; H, 12.98. Found: C, 77.24; H, 12.93.

*Identification of (2*R,*3*R*)-3-hydroxy-2-tetradecyloctadecanoic acid* [(+)**4a**]. — Compound **11a** (50 mg) was suspended in 15% aqueous tetrabutylammonium hydroxide (2 mL) and heated overnight at 100° (ref. 30). The hydrolyzate was acidified with M HCl (litmus paper) and the corynomycolic acid was extracted with petroleum ether. It cochromatographed with (±)**4a** in 19:1 chloroform–methanol and in 200:200:1 diethyl ether–hexane–acetic acid, and when crystallized from ethanol had m.p. 69–70°; lit.[4] 69–70°; $[\alpha]_D^{20}$ +6.9° (*c* 0.55, chloroform); lit.[4] +7.5°.

*Identification of (2*S,*3*S*)-3-hydroxy-2-tetradecyloctadecanoic acid* [(−)**4a**]. — Saponification of **11b**, as just described for **11a**, yielded (−)**4a**. This isomer also cochromatographed with (±)**4a** in 19:1 chloroform–methanol and in 200:200:1 diethyl ether–hexane–acetic acid. It had m.p. 68–70° (from ethanol) and $[\alpha]_D^{20}$ −7.1° (*c* 0.6, chloroform).

*(2*RS,*3*SR*)-3-Hydroxy-2-tetradecyloctadecanoic acid* (**4b**). — Compound **3b** was saponified, as described for **3a**, to afford **4b**. After crystallization from ethanol, the compound melted at 75–77°, lit.[15] 73–75°.

Anal. Calc. for $C_{32}H_{64}O_3$ (496.86): C, 77.36; H, 12.98. Found: C, 76.73; H, 12.97.

*(2*RS,*3*RS*)-3-(*tert*-Butyldimethylsilyloxy)-2-tetradecyloctadecanoic acid* (**5a**). — A solution of imidazole (1.1 g, 16 mmol) and *tert*-butylchlorodimethylsilane (904 mg, 6 mmol) in dry *N,N*-dimethylformamide (10 mL) was added to **4a** (1 g, 2 mmol) in dry toluene (15 mL). This mixture was heated overnight at 75° and extracted with three 50-mL portions of hexane. The hexane layers were passed through a column (1.1 × 8 cm) of neutral alumina prewashed with diethyl ether[16], the column was washed with petroleum ether (b.p. 37–53°, 100 mL), and the eluates were pooled and evaporated to dryness. T.l.c. in chloroform showed three major spots for the silylated products (see Discussion). The material was dissolved in chloroform (50 mL), the solution was stirred with 10% aq. K_2CO_3 solution (25 mL) at room temperature for 15–20 min to cleave silyl ester groups[31], and then the mixture was acidified with $KHSO_4$. The product (**5a**) was extracted with chloroform, and the chloroform solution was dried (Na_2SO_4), filtered, and concentrated to dryness. Purification of the residual material over a column (2.1 × 27 cm) of Silica Gel 60 (elution with chloroform) gave syrupy **5a** (980 mg, 80%), single spot (R_F 0.62) on t.l.c. in chloroform; ^{1}H-n.m.r. ($CDCl_3$): δ 3.9 (q, 1 H, H-3), 2.57 (m, 1 H, H-2), 1.7–1.45 (m, CH_2), 1.42–1.20 (m, CH_2), 0.92 (m, terminal CH_3 and CCH_3 of Bu^t), and 0.10 (m, $SiCH_3$); e.i.m.s. of the methyl ester (diazomethane): *m/z* 624 (M^+), 610 (M

$- CH_3 + H^+$), 567 ($M - C_4H_9$), and 355 (M − methyl palmitate + H^+).

Anal. Calc. for $C_{38}H_{78}O_3Si$ (611.13): C, 74.68; H, 12.87. Found: C, 74.67; H, 12.54.

*(2RS,3SR)-3-(*tert*-Butyldimethylsilyloxy)-2-tetradecyloctadecanoic acid* (**5b**). — Compound **4b** (400 mg, 0.8 mmol) was derivatized to **5b** and purified as just described for **5a**; ^{1}H-N.m.r. ($CDCl_3$): δ 3.9 (q, 1 H, H-3), 2.49 (m, 1 H, H-2), 1.72 (m, CH_2), 1.5 (m, CH_2), 1.45 (m, CH_2), 1.37–1.13 (m, CH_2), 1.0–0.81 (m, terminal CH_3 and CCH_3 of Bu^t), and 0.10 (m, $SiCH_3$); e.i.m.s. of the methyl ester (diazomethane) similar to that of **5a**.

Anal. Calc. for $C_{38}H_{78}O_3Si$ (611.13): C, 74.68; H, 12.87. Found: C, 74.82; H, 12.34.

*2,3,4,6,2',3',4',6'-Octakis-*O*-(trimethylsilyl)-α,α-trehalose* (**6**). — Compound **6** was prepared from trehalose dihydrate (2.0 g, 5.3 mmol) following the method of Toubiana *et al.*[17]. After elimination of solvents, t.l.c. in 19:1 hexane–diethyl ether showed a single band at R_F 0.45. The yield of **6** was 4.4 g (90%); on crystallization from methanol, it had m.p. 80–82°, $[\alpha]_D^{20}$ +94° (*c* 1.5, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ 94.69 (C-1), 73.91, 73.57, 73.22, 72.05, and 62.43 (C-6); lit.[17] m.p. 80–82°, $[\alpha]_D$ +95°.

*2,3,4,2',3',4',6'-Heptakis-*O*-(trimethylsilyl)-α,α-trehalose* (**7**). — Compound **6** (2.1 g, 2.3 mmol) was kept in 0.2% methanolic K_2CO_3 for ~20 min at 0–4° (*cf.* ref. 18). After neutralization with acetic acid (4.5 mL), the reaction mixture was partitioned between chloroform (200 mL) and water (120 mL), and the upper layer was washed with cold chloroform. The combined chloroform extracts were washed twice with the upper layer of a 10:5:6 chloroform–methanol–water mixture, dried (Na_2SO_4), and concentrated. The residue, suspended in petroleum ether, was loaded onto a column of silica gel, and the components were separated by stepwise elution with petroleum ether–diethyl ether (12:1, 3:1, and 1:3). Evaporation of the second fraction gave 1.26 g (65%) of **7**, single spot (R_F 0.4) on t.l.c. in 4:1 petroleum ether–diethyl ether. The recrystallized **7** (from 9:1 acetonitrile–methanol) showed m.p. 76–78°, $[\alpha]_D^{20}$ +114.5° (*c* 2.3, pet. ether); ^{1}H-n.m.r. ($CDCl_3$): δ 4.92, 4.87 (2 d, 2 H, *J* 3.1 Hz, H-1,1'), 3.89 (t, 2 H, *J* 8.5 Hz), 3.82, 3.77 (2 dt, 2 H), 3.66 (~s, br., 4 H), 3.47 (t, 1 H, *J* 10 Hz), and 3.43–3.34 (m, 3 H); ^{13}C-n.m.r. ($CDCl_3$): δ 94.80, 94.64 (C-1,1'), 73.76, 73.16, 71.94, 71.73, 62.34 (C-6'), and 61.76 (C-6); lit.[18] m.p. 76–78°, $[\alpha]_D^{18}$ +115°.

Anal. Calc. for $C_{33}H_{78}O_{11}Si_7$ (847.58): C, 46.76; H, 9.28. Found: C, 46.88; H, 9.31.

After the addition of trichloroacetyl isocyanate to the sample, the ^{1}H-n.m.r. spectrum ($CDCl_3$) showed: δ 4.97, 4.91 (2 d, 2 H, *J* 2.7 Hz, H-1,1'), 4.53 (br. d, 1 H, *J* 10.4 Hz, H-6a)*, 4.26 (dd, 1 H, *J* 4.7, 11.5 Hz; →t, *J* 4.2 Hz on irrad. at 4.53; →d, *J* 10.8 Hz on irrad. at 4.07, H-6b), 4.07 (m, 1 H, →dd, *J* 4.6, 9.5 Hz on irrad. at 4.53; →br. s on irrad. at 3.54, H-5), 3.93 (two overlapping t's, 2 H, *J* 9.0 Hz), 3.78 (m, 1 H), 3.70 (d, *J* 3.0 Hz, or narrow m, 2 H), 3.54 (t, 1 H, *J* 9.0 Hz; →d, *J* 8.1 Hz on irrad. at 4.07, H-4), 3.47 (m, 2 H), and 3.41 (dd, 1 H, *J* 2.8, 9.3 Hz).

*2,3,4,2',3',4'-Hexakis-*O*-(trimethylsilyl)-α,α-trehalose* (**8**). — Compound **8** was obtained from **6** (1.0 g, 1.1 mmol) as described by Toubiana *et al.*[17]. After purification, the yield of **8** was ~750 mg (~90%). The recrystallized **8** (from methanol) showed R_F

* Of the two protons at each of the 6-positions, the one resonating at lower field is designated H-6a or -6'a, the other H-6b or -6'b.

0.28 in 1:1 petroleum ether–diethyl ether; m.p. 116–118°, $[\alpha]_D^{20}$ +102° (*c* 2.35, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 4.92 (d, 2 H, *J* 3.1 Hz, H-1,1'), 3.91–3.85 (m), 3.67 (m), 3.47 (t, 2 H, *J* 9.1 Hz), and 3.43 (dd, 2 H, *J* 3.1, 9.3 Hz); ^{13}C-n.m.r. ($CDCl_3$): δ 94.57 (C-1,1'), 73.30, 73.26, 72.70, 71.28, and 61.24 (C-6,6'); lit.[17] 115–118°, $[\alpha]_D$ +100°.

Anal. Calc. for $C_{30}H_{70}O_{11}Si_6$ (775.40): C, 46.47; H, 9.10. Found: C, 46.07; H, 9.31.

Conversion of **8** *into monoester* **11**. — Following the general procedure for acylation, compound **8** (811 mg, 1.05 mmol) was coupled with **5a** (640 mg, 1.05 mmol) in toluene (3 mL) in the presence of 1,3-dicyclohexylcarbodiimide (220 mg, 1.07 mmol) and 4-dimethylaminopyridine (~15 mg). T.l.c. with 17:3 hexane–ether showed strong spots at R_F 0.54 and 0.38 for the diastereomeric components (**11a**, **11b**) of the monoester. Plates developed with chloroform exhibited a weak spot for the diester **12** at R_F ~0.95, and strong spots at 0.27 (**11b**) and 0.23 (**11a**). Chromatography of the mixture on a column of neutral alumina (3 × 42 cm, elution with 19:1 hexane–ether) gave first a small amount (~20 mg) of **12**, then the monoester **11**, partially separated into a leading fraction (**11a**), a center cut (mixture, ~65% of the total), and a trailing fraction (**11b**). Concentration of the monoester fractions to dryness left residues, probably retaining some solvent, having a total weight of 1.2 g. The mixture of **11a** and **11b** was a light-yellow syrup, $[\alpha]_D^{20}$ +59° (*c* 2.5, chloroform); its ^{1}H-n.m.r. spectrum was the sum of the spectra of **11a** and **11b** (see next).

*6-O-[(2R,3R)-3-(*tert*-Butyldimethylsilyloxy)-2-tetradecyloctadecanoyl]-2,3,4,-2',3',4'-hexakis-O-(trimethylsilyl)-α,α-trehalose* (**11a**). — Rechromatography of a portion (160 mg) of the material from the foregoing center cut gave pure **11a** (74 mg), $[\alpha]_D^{20}$ +62° (*c* 4.8, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 4.90, 4.83 (2 d, *J* 3.0 Hz, H-1,1'), 4.34 (dd, *J* 2.2, 11.7 Hz, H-6a), 4.22 (sept, H-5), 4.07 (dd, *J* 4.0, 11.8 Hz, H-6b), 4.03–3.30 (series of m, sugar CH, H-3 of acyl), 2.55 (m, 1 H, H-2 of acyl), 1.80–1.00 (m, CH_2 of acyl), 0.98–0.70 (m, terminal CH_3, $SiCCH_3$), and 0.38–0.00 (m, $SiCH_3$).

Anal. Calc. for $C_{68}H_{146}O_{13}Si_7$ (1368.51): C, 59.68; H, 10.75. Found: C, 59.85; H, 10.65.

*6-O-[(2S,3S)-3-(*tert*-Butyldimethylsilyloxy)-2-tetradecyloctadecanoyl]-2,3,4,-2',3',4'-hexakis-O-(trimethylsilyl)-α,α-trehalose* (**11b**). — Continued elution gave **11b** (78 mg), $[\alpha]_D^{20}$ +58° (*c* 1.8, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 4.89, 4.82 (2 d, 2 H, *J* 3.1 Hz, H-1,1'), 4.48 (~dd, *J* ~1, 9.5 Hz, H-6a), 3.98–3.31 (series of m, sugar CH and CH_2, H-3 of acyl), 2.55 (m, 1 H, H-2 of acyl), 1.80–1.06 (m, CH_2 of acyl), 0.98–0.80 (m, terminal CH_3, $SiCCH_3$), and 0.38–0.00 (m, $SiCH_3$).

Anal. Calc. for $C_{68}H_{146}O_{13}Si_7$ (1368.51): C, 59.68; H, 10.75. Found: C, 59.15; H, 10.74.

*6-O-[(2RS,3SR)-3-(*tert*-Butyldimethylsilyloxy)-2-tetradecyloctadecanoyl]-α,α-trehalose* (**15**). — Compounds **5b** (268 mg, 0.44 mmol) and **7** (370 mg, 0.44 mmol) were coupled (see *Acylation*) in toluene (2.0 mL) with the aid of 1,3-dicyclohexylcarbodiimide (90 mg, 0.44 mmol) and 4-dimethylaminopyridine (10 mg). The product was purified on a column of silicic acid eluted with 19:1 hexane–diethyl ether to yield **10** (493 mg, 78%), $[\alpha]_D^{20}$ +34.5° (*c* 1.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 4.85, 4.82 (d and prob. 3 overlapping d, 2 H, *J* ~2.8 Hz, H-1,1'), 4.61, 4.46 (~dd, *J* ~1, 11.8 Hz, and br. d, *J*

11.8 Hz, 1 H, H-6a of the resp. diastereomers), 3.98–3.27 (6 m, H-6'a,6b,6'b and CH of the sugar, H-3 of acyl), 2.50 (m, 1 H, H-2 of acyl), 1.23, 0.86 (br. s and narrow m, CH_2 and CH_3 groups of the acid, and $SiCCH_3$), and 0.02–0.00 (m, $SiCH_3$).

A solution of **10** (150 mg) in 80% aqueous acetic acid (5 mL) was heated under reflux for 30 min, and then evaporated to dryness. The residue was transferred to a column (1 × 40 cm) of silica gel prepared in chloroform, and the column was eluted successively with 9:1 (240 mL) and then 4:1 chloroform–methanol. This gave pure **15** (60 mg, 62%), $[\alpha]_D^{20}$ +70° (*c* 2, chloroform); the californium plasma desorption m.s. showed positive ions at *m/z* 1892.7 ($M_2 + Na^+$), 1835.7 ($M_2 + Na^+ - C_4H_9$), 958.9 ($M + Na^+$, calc. 957.7), and 878.2 ($M + H^+ - C_4H_9$), and negative ions at *m/z* 1851.2 ($M_2 - H_2O - H^+$, calc. 1850.3) and 971.6 ($M + Cl^-$, calc. 969.7); positive-ion fast-atom bombardment m.s.: *m/z* 957.6 ($M + Na^+$), 935.6 ($M + H^+$), 877.5 ($M + H - C_4H_9$, calc. 878.6), 773.5, and 593.6 (silylated corynomycoloyl).

Anal. Calc. for $C_{50}H_{98}O_{13}Si$ (935.41): C, 64.19; H, 10.56. Found: C, 63.81; H, 10.60.

6-O-[(2RS,3SR)-3-Hydroxy-2-tetradecyloctadecanoyl]-α,α-trehalose (**16**). — Compound **10** (300 mg) was prepared as just described, and desilylated as detailed under *Deprotection of the coupling products*, above. The desilylated material was purified on a silicic acid column (2.4 × 32 cm), successively eluted with 9:1 (200 mL), 22:3 (200 mL), and 17:3 chloroform–methanol. The yield of **16** showing a single spot at R_F 0.4 in 39:11:1 chloroform–methanol–water was 138 mg (79%), m.p. 122–124° (deposited from methanol), $[\alpha]_D^{20}$ +84° (*c* 2.2, chloroform); ^{1}H-n.m.r. [($^2H_6$)Me_2SO_2 + trifluoroacetic acid]: δ 4.85 (d, 2 H, *J* 3.1 Hz, H-1,1'), 4.3 (t, 1 H, *J* 9.5 Hz, H-6a acylated), 4.03–3.95 (m, 1 H), 3.92–3.86 (m, 1 H), 3.67–3.61 (m, 1 H), 3.59–3.08 (series of m), 2.22 (m, 1 H, H-2 of acyl), 1.6–1.09 (m, CH_2 of acyl), 1.07, and 0.85 (2 t, 6 H, terminal CH_3); californium plasma desorption m.s.: positive ion, *m/z* 843 ($M + Na^+$), negative ion, *m/z* 856 ($M + Cl^-$).

Anal. Calc. for $C_{44}H_{84}O_{13}\cdot H_2O$ (839.16): C, 62.98; H, 10.33. Found: C, 62.58; H, 10.24.

6-O-[(2RS,3RS)-3-Hydroxy-2-tetradecyloctadecanoyl]-α,α-trehalose (**17**) *and its diastereomeric components* **18** *and* **19**. — The deprotection of **11** (200 mg) by the standard procedure yielded **17**, which was purified on a column of silicic acid (2.4 × 30 cm, successive elutions with 9:1, 22:3, and 17:3 chloroform–methanol). The purified material (91 mg, 74%) was a white solid having R_F 0.4 in 39:11:1 chloroform–methanol–water and cochromatographing with **16** and also with trehalose monocorynomycolate in mixed esters from *C. diphtheriae*. It had m.p. 195–198° (deposited from methanol) and $[\alpha]_D^{20}$ +60° (*c* 0.69, chloroform); ^{1}H-n.m.r. [($^2H_6$)Me_2SO + trifluoroacetic acid]: δ 4.85 (d, 2 H, *J* 3.7 Hz, H-1,1'), 4.33 (br. d, 1 H, *J* 10.9 Hz, H-6a), 3.98 (br. dd, 1 H, *J* 5.0, 11.5 Hz, H-6b), 3.92–3.86 (m, 1 H), 3.67–3.61 (m, 1 H), 3.60–3.10 (series of m), 2.30 (m, 1 H, H-2 of acyl), 1.56 (m), 1.45–1.15 (m, CH_2 of acyl), 0.93, and 0.85 (2 t, 6 H, terminal CH_3); californium plasma desorption m.s.: positive ion, *m/z* 843 ($M + Na^+$); lit.[32] $[\alpha]_D^{24}$ +76.5°.

Anal. Calc. for $C_{44}H_{84}O_{13}\cdot H_2O$ (839.16): C, 62.98; H, 10.33. Found: C, 62.83; H, 10.24.

Similarly, compound **11a** (70 mg) was desilylated and the product was purified by column chromatography to yield 34 mg of **18**, which cochromatographed with **17** and melted at 195–196° (deposited from methanol), $[\alpha]_D^{20}$ +68° (*c* 0.5, chloroform); ^{1}H-n.m.r. [($^2H_6$)Me_2SO + trifluoroacetic acid]: δ 4.87 (d, 2 H, *J* 2.8 Hz, H-1,1'), 4.30 (d, 1 H, *J* 11.5 Hz, H-6a), 4.02 (dd, 1 H, *J* 5.3, 11.6 Hz; →d, *J* 4.4 Hz on irrad. at 4.30; →d, *J* 11.5 Hz on irrad. at 3.93, H-6b), 3.93 (m, 1 H; →dd on irrad. at 4.30, H-5), 3.67 (m, 1 H, prob. H-5'), 3.62–3.10 (series of m), 2.31 (m, H-2 of acyl), 1.60–1.05 (m, CH_2 of acyl), and 0.89 (t, 6 H, terminal CH_3).

Compound **11b** (20 mg) was similarly desilylated to yield, after chromatography on silicic acid, **19** (8 mg). This product cochromatographed with **18**, and had m.p. 195–197° (deposited from methanol); $[\alpha]_D^{20}$ +55° (*c* 0.3, chloroform); its ^{1}H-n.m.r. [($^2H_6$)Me_2SO + trifluoroacetic acid] was similar to that of **18**.

6-O-Palmitoyl-α,α-trehalose (**20**). — Palmitic acid (153 mg, 0.60 mmol) and **8** (462 mg, 0.60 mmol) were esterified in the presence of 1,3-dicyclohexylcarbodiimide (123 mg, 0.60 mmol) and 4-dimethylaminopyridine (~10 mg) in dry dichloromethane (2.5 mL). The general procedure for acylation was followed, except that the reaction was allowed to go to completion at room temperature (overnight). A part (~5 mg) of the crude product was purified on four 20 × 20 cm t.l.c. plates (Fisher Scientific) (irrigation with chloroform) to give **13** showing a single spot, R_f 0.23 in chloroform and 0.14 in 17:3 hexane–ether; ^{1}H-n.m.r. ($CDCl_3$): δ 4.91 (two overlapped d's, 2 H, *J* 3.0 Hz, H-1,1'), 4.29 (dd, 1 H, *J* 1.5, 10.4 Hz, H-6a), 4.06 (dd, 1 H, *J* 4.4, 11.8 Hz, H-6b), 4.00 (m, 1 H, H-5), 3.93–3.38 (series of m, sugar CH and CH_2), 2.35 (m, α-CH_2 of acyl), 1.74, 1.62 (~t and m, β-CH_2 of acyl?), 1.25 (m, CH_2 of acyl), 0.89 (t, terminal CH_3), and 0.11 (m, $SiCH_3$).

The remaining **13** was hydrolyzed by treatment with 8:17:3 trifluoroacetic acid–oxolane–water for ~1 h at room temperature, and the product was chromatographed on a column of silicic acid (2.2 × 23 cm), eluted first with 9:1, then 22:3, and finally 4:1 chloroform–methanol. Contaminating salts were removed by passing the compound in 4:1 chloroform–methanol through a small column (1 × 15 cm) packed with Chelex 100 (Na^+) layered over Dowex 50-X8 (H^+) cation-exchange resins, and concentrating the effluent. The purified product **20** (245 mg; 68%) showed a single spot, R_f 0.25, on t.l.c. in 39:11:1 chloroform–methanol–water. It crystallized from acetone, softening on the micro-block from 132 to 140° and melting* at 198–200°, $[\alpha]_D^{20}$ +68° (*c* 1, methanol); ^{1}H-n.m.r. [($^2H_6$)Me_2SO + trifluoroacetic acid]: δ 4.85, 4.82 (2 d, 2 H, *J* 3.5 Hz, H-1,1'), 4.21 (br. d, 1 H, *J* 11.1 Hz, H-6a), 4.02 (dd, 1 H, *J* 5.5, 11.6 Hz, H-6b), 3.91–3.85 (m, 1 H, H-5), 3.67–3.60 (m, 1 H, H-5'), 3.58–3.08 (series of m, sugar CH and CH_2), 2.25 (t, α-CH_2 of acyl), 1.48 (m, β-CH_2 of acyl), 1.35–1.17 (m, acyl CH_2), and 0.84 (t, terminal CH_3).

Anal. Calc. for $C_{28}H_{52}O_{12}\cdot 1.5H_2O$ (607.73): C, 55.34; H, 9.12. Found: C, 55.38; H, 9.06.

6,6'-Di-O-[(2RS,3RS)-3-hydroxy-2-tetradecyloctadecanoyl]-α,α-trehalose (**21**). — Compounds **5a** (167 mg, 0.27 mmol) and **8** (85 mg, 0.11 mmol) were coupled in the presence of 1,3-dicyclohexylcarbodiimide (56 mg, 0.27 mmol) and 4-dimethylaminopy-

* An earlier preparation[33] having a much lower m.p. (117–118°) may have been a mixture of regioisomers.

ridine (~5 mg) by use of the standard procedure for acylation. The product was chromatographed on a column (3.4 × 32 cm) of silica gel eluted first with 49:1 (600 mL), and then 19:1 hexane–ether, to yield **12** (149 mg, 69%), oil, $[\alpha]_D^{20}$ +15.5° (*c* 1.45, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 4.82 (m, H-1,1'), 4.50, 4.31 (dd, br. dd, H-6a,6'a), 4.21 (envelope, prob. H-5,6b,5',6'b), 4.31–4.01 (sugar CH and CH_2, H-3 of acyl), 3.33, 3.32 (2 overlapping t, *J* 9.3 Hz), 3.0 (t, 3 H), 2.55 (m, 1 H, H-2 of acyl), 2.32–1.00 (m, CH_2 of acyl), 0.98–0.70 (m, terminal CH_3, $SiCCH_3$), and 0.31–0.00 (m, $SiCH_3$).

The desilylation of **12** (100 mg) by the standard procedure, and chromatography of the product on a column of silicic acid (2.2 × 21 cm) eluted successively with chloroform (200 mL), 19:1 (200 mL), and 9:1 chloroform–methanol gave **21** (41 mg, 61%), single spot, R_F 0.78, on t.l.c. in 39:11:1 chloroform–methanol–water, m.p. (deposited from methanol) 150–151°, $[\alpha]_D^{20}$ +42° (*c* 1, chloroform); ^{1}H-n.m.r. [($^2H_6$)-Me_2SO + trifluoroacetic acid]: δ 4.86 (br., 2 H, H-1,1'), 4.37, 4.29 (2 br. d, H-6a and/or 6'a), 4.06–2.96 (series of m, sugar CH and CH_2, H-3 of acyl), 2.28 (m, 2 H, H-2 of acyl), 1.65–1.05 (series of m, acyl CH_2), 0.93, and 0.86 (2 t, terminal CH_3); laser desorption m.s.: positive ion, *m/z* 1322 ($M + Na^+$); lit.[25] $[\alpha]_D$ +51.4°.

Anal. Calc. for $C_{76}H_{146}O_{15}\cdot H_2O$ (1318.00): C, 69.26; H, 11.32. Found: C, 68.93; H, 11.28.

6,6'-Di-O-palmitoyl-α,α-trehalose (**22**). — Palmitic acid (154 mg, 0.60 mmol) was coupled with **8** (154 mg, 0.20 mmol) in the presence of 1,3-dicyclohexylcarbodiimide (123 mg, 0.60 mmol) and 4-dimethylaminopyridine (~10 mg) in dry dichloromethane (2 mL), according to the general procedure for acylation modified as described for **20**. A part (~6 mg) of the coupling product (**14**) was chromatographed on four 20 × 20 cm t.l.c. plates (Fisher Scientific) (irrigation with 19:1 hexane–ether), R_F 0.45 in chloroform and in 17:3 hexane–ether; ^{1}H-n.m.r. ($CDCl_3$): δ 4.91 (d, 2 H, *J* 3.0 Hz, H-1,1'), 4.27 (dd, 2 H, *J* 2.0, 11.8 Hz, H-6a,6'a), 4.05 (dd, 2 H, *J* 4.4, 11.8 Hz, H-6b,6'b), 4.00 (m, H-5,5'), 3.90 (t, 2 H, *J* 9.0 Hz, H-3,3' or 4,4'), 3.47 (t, 2 H, *J* 9.1 Hz, H-4,4' or 3,3'), 3.43 (dd, 2 H, *J* 3.1, 9.3 Hz, H-2,2'), 2.32 (m, α-CH_2 of acyl), 1.6 (m, β-CH_2 of acyl), 1.37–1.15 (m, acyl CH_2), 0.89 (t, terminal CH_3), and 0.11 (m, $SiCH_3$).

The remaining reaction product was dissolved in 8:17:33 trifluoroacetic acid–oxolane–water and kept at room temperature until t.l.c. showed the hydrolysis to be complete (~1 h). The product, chromatographed on a silicic acid column (2.2 × 21 cm) eluted as described for compound **21**, gave **22** (106 mg, 64% based on **8**), single spot, R_F 0.62 in 39:11:1 chloroform–methanol–water, m.p. (deposited from hexane) 155–158°, $[\alpha]_D^{20}$ +80° (*c* 1.2, chloroform); ^{1}H-n.m.r. [($^2H_6$)Me_2SO + trifluoroacetic acid]: δ 4.81 (d, 2 H, *J* 3.5 Hz, H-1,1'), 4.21 (br. d, 2 H, *J* 10.4 Hz, H-6a,6'a), 4.02 (dd, 2 H, *J* 5.7, 11.7 Hz, H-6b,6'b), 3.87 (m, 2 H, H-5,5'), 3.53 (t, 2 H, *J* 9.1 Hz, H-3,3' or 4,4'), 3.24 (dd, 2 H, *J* 3.6, 9.5 Hz, H-2,2'), 3.10 (t, 2 H, *J* 9.3 Hz, H-4,4' or 3,3'), 2.26 (t, α-CH_2 of acyl), 1.50 (m, β-CH_2 of acyl), 1.30–1.15 (m, acyl CH_2), and 0.84 (t, terminal CH_3); lit.[17] m.p. 154–158°, $[\alpha]_D$ +80°.

Anal. Calc. for $C_{44}H_{82}O_{13}\cdot 0.5H_2O$ (828.13): C, 63.82; H, 10.10. Found: C, 63.74; H, 10.24.

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