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Synthesis and biological evaluation of trehalose analogs as potential inhibitors of mycobacterial cell wall biosynthesis

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Abstract

Analogs of trehalose are reported that were designed to interfere with mycolylation pathways in the mycobacterial cell wall. Several derivatives of 6,6′-dideoxytrehalose, including *N*,*N*′-dialkylamino and 6,6′-bis(sulfonamido) analogs, were prepared and evaluated for antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Ra$ and a panel of clinical isolates of *Mycobacterium avium*. 6,6′-Diaminotrehalose and its diazido precursor were both inactive, but significant activity apparently related to aliphatic chain length was found among the sulfonamides, *N*-alkylamines, and one of the amidines.

Keywords: Trehalose mono- and di-mycolate; Mycolylation; Antitubercular agents; Antigen 85 complex

1. Introduction

Tuberculosis, although nominally a curable disease, remains one of the primary health threats worldwide.[1,2] The appearance of drug-resistant, particularly multiple drug-resistant, forms of the disease throughout the world has lead to the realization that tuberculosis may again become an incurable disease.[3–10] In fact, for some multiple drug-resistant strains, there are now few treatment options.[11] World health officials are calling for a concerted effort to understand the biochemistry and pathology of the bacterium that may lead to the development of new, more effective, and safer vaccines and drugs.[12,13] In particular, new drugs with novel mechanisms of action will be required to treat drug-resistant forms of tuberculosis.[14–16]

One of the chief distinguishing characteristics of mycobacteria is a thick lipid envelope, the various constituents of which are bound to an arabinogalactan polysaccharide coat through both covalent and non-covalent forces.[17] The mycolic acids are a unique group of functionalized, very long chain (>60 carbon atoms), α-branched, β-hydroxylated carboxylic acids that are esterified to terminal arabinan branches of this polysaccharide, thus forming the core of the lipid envelope.[18] Since one of the more effective antituberculosis drugs, isoniazid, acts through inhibition of mycolic acid biogenesis,[19] and since the lipid layer itself is thought to hinder uptake of other potential drugs, disruption of the mycolate structure is a compelling strategy for the development of novel agents.

Trehalose is vitally important to the mycolate economy of mycobacteria.[20] Both 6-mycolyltrehalose and 6,6′-dimycolyltrehalose are believed to act as donors for mycolate transfer to arabinan during envelope elaboration. Recently, it was reported[21] that the antigen 85 complex (ag85 A, B, and C) appears to play a role in cell-wall biogenesis, and, in particular these proteins possess a mycolyltransferase capability that utilizes trehalose mono- and di-mycolates. Our laboratories have synthesized a series of trehalose derivatives designed to inhibit this mycolyltransferase activity. In the design of these agents, we reasoned that for transesterification to proceed efficiently there would likely be an activating

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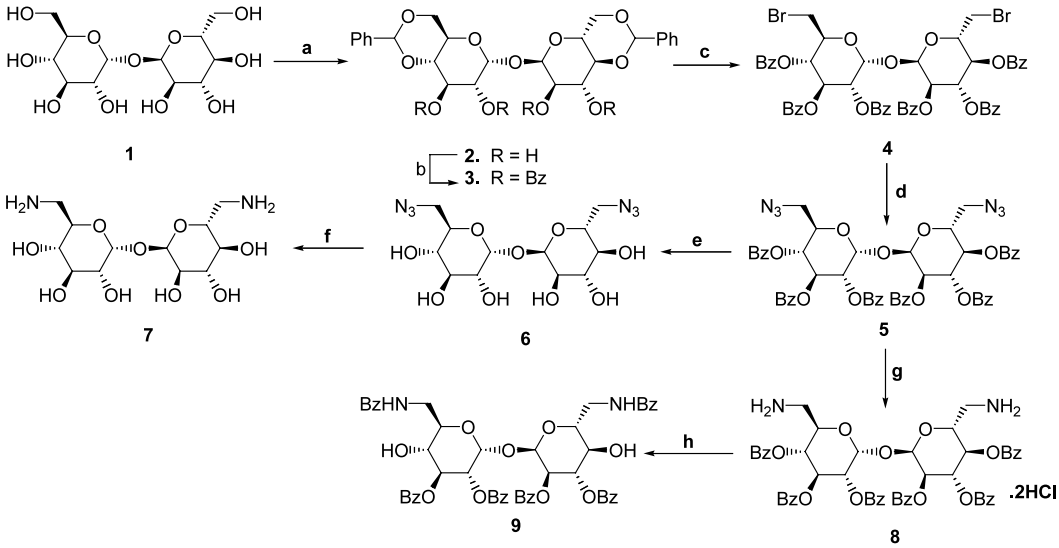


Scheme 1. Reagents and conditions: (a) C_6H_5CHO, $ZnCl_2$, rt, 6 days; (b) C_6H_5COCl, pyridine, rt, 64 h; (c) NBS, K_2CO_3, CCl_4, reflux, 2 h; (d) NaN_3, CH_3OH, rt, 48 h; (e) $NaOCH_3$, CH_3OH, rt, 48 h, cation resin exchange H^+ form; (f) H_2, 5% Pd–C, MeOH, 28 h; (g) H_2, Pd–C, EtOH, HCl; (h) $NaHCO_3$–H_2O or **g** without additon of HCl.

moiety, perhaps an aspartate or glutamate residue and/or possibly a basic function such as a histidine that could catalyze the reaction through a general acid–base mechanism. This activation is probable irrespective of whether an enzyme-bound intermediate is present. Though the precise mechanism of mycolyl transfer was not known at the inception of our program, a recent publication[21] of the crystal structure of ag85C at 1.5 Å reveals a catalytic triad formed by Ser 124, Glu 228, and His 260, reinforcing our original hypothesis. Consequently, analogs with the carbonyl replaced by a complementary basic group (amine or amidine) that could interact with an acidic function through a salt bridge were targeted. Furthermore, acidic sulfonamide analogs that could take advantage of a basic function within the active site were also prepared. Finally, a hydrophobic group that could partially occupy the mycolate binding pocket was considered essential, since it is well known that hydrophobic forces typically provide most of the ligand binding energy.[22]

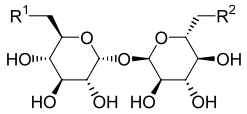
Several derivatives of 6,6′-diamino-6,6′-dideoxytrehalose (**7**), including *N*,*N*′-dialkylamino (compounds **12**, **15a**–**d**, **18a**–**c**, and **21**) and 6,6′-bis(sulfonamido) (compounds **11a**–**d**), were prepared and evaluated for antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Ra$ and a panel of clinical isolates of *Mycobacterium avium*.

2. Results and discussion

Preparation of key intermediates.—Our initial synthetic goal was to prepare representatives of several classes of 6,6′-bis(substituted-amino)-6,6′-dideoxy-α,α-trehalose derivatives: sulfonamides, alkylamines, amidines, and guanidine, and to use biological assay for antimycobacterial activity to guide further synthetic efforts. 6,6′-Diamino-6,6′-dideoxytrehalose (**7**), a potentially useful starting material, has been obtained previously[23] by tosylation of anhydrous α,α-trehalose followed by direct displacement with ammonia, or in three steps by the sequence 6,6′-ditosylate → 6,6′-diazido → 6,6′-diamine. However, in a single pilot experiment, tosylation gave a complex mixture of products that required tedious column separation and gave a low yield of the desired component, a result that has been confirmed by others.[24,25]

A better route that allowed synthesis of **7** in multigram quantities is outlined in Scheme 1. It uses well-documented methodology and, with trivial variations, is adaptable to either the benzyl- or benzoyl-protected sugar. In addition, Scheme 1 afforded the 6,6′-diazido compound **6**, which we wanted for bioassay, as well as compounds **4** and **8**, both of which saw limited utility as starting materials for some of the target compounds in Table 1. Anhydrous α,α-trehalose (**1**) was obtained from the commercially available dihydrate either by prolonged drying in vacuo at 78 °C[26] or by azeotropic distillation with pyridine.[27] Treatment of **1** with freshly distilled benzaldehyde–anhydrous $ZnCl_2$ followed by perbenzoylation gave **2**[26] and **3**[28–30] in yields of 98 and 80%, respectively. The conversion of **3** into **4** in Scheme 1 followed essentially the procedures given by Hanessian.[29] The only significant departure was the substitution of anhydrous K_2CO_3 for $BaCO_3$ in step (c) leading to **4**. Also, isopropyl alcohol was substituted for acetone–ether–pentane as recrystallization solvent. Both methods gave ~80% purified yield of **4**. Since adequate

Table 1

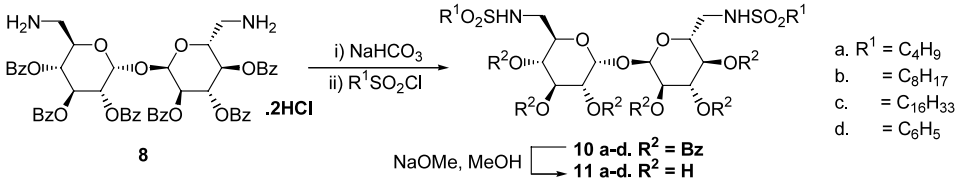


No.	SRI no.	R^1 and R^2	MIC (μg/mL) [a]						
			MTB H37Ra	MAV NJ168	MAV NJ211	MAV NJ1854	MAV NJ3009	MAV NJ3350	MAV NJ3404
6	8633	$R^1 = R^2 = N_3$	ND [b]	>128	ND	ND	ND	>128	>128
7	8998	$R^1 = R^2 = NH_2$	ND	>128	ND	ND	ND	ND	>128
11a	8706	$R^1 = R^2 = CH_3(CH_2)_3SO_2NH–$	>128	>128	>128	ND	ND	ND	>128
11b	8667	$R^1 = R^2 = CH_3(CH_2)_7SO_2NH–$	16–32	32–64	64	32–64	32–64	16–32	32–64
11c	8648	$R^1 = R^2 = CH_3(CH_2)_{15}SO_2NH–$	>32	>64	>32	ND	ND	>64	>64
11d	8649	$R^1 = R^2 = C_6H_5SO_2NH–$	ND	>128	ND	ND	ND	>128	>128
12	8855	$R^1 = R^2 = [CH_3(CH_2)_{11}]_2N–$	>128	>128	>128	>128	>128	ND	>128
15a	8792	$R^1 = R^2 = CH_3(CH_2)_7NH–$	4	8	2	ND	2	ND	4
15b	8793	$R^1 = R^2 = CH_3(CH_2)_{11}NH–$	8	8	8	ND	8	ND	8
15c	8982	$R^1 = R^2 = CH_3(CH_2)_{17}N(CH_3)–$	>128	>128	>128	ND	ND	ND	>128
15d	8980	$R^1 = R^2 = [CH_3(CH_2)_3CH(Et)CH_2]_2N–$	128	128	8	>128	ND	ND	64
18a	9009	$R^1 = R^2 = CH_3(CH_2)_5NH–$	128	>128	128	ND	ND	ND	>128
18b	9029	$R^1 = R^2 = CH_3(CH_2)_9NH–$	>1.3≤13	>1.3≤13	>1.3≤13	ND	ND	ND	>1.3≤13
18c	8979	$R^1 = R^2 = [CH_3(CH_2)_3CH(Et)CH_2NH–$	32	64	16	32	ND	ND	64
21	9046	$R^1 = CH_3(CH_2)_3CH(Et)CH_2NH–$; $R^2 = OH$	>128	>128	>128	ND	ND	ND	>128
24,25	8849	$R^1 = R^2 = CH_3(CH_2)_4C(NH)NH–$, mixture with ~10% [$R^1 = CH_3(CH_2)_4C(NH)NH–$ and $R^2 = CH_3(CH_2)_4CONH–$]	>128	>128	>128	ND	ND	ND	>128
26	8984	$R^1 = NH_2$; $R^2 = CH_3(CH_2)_{10}C(NH)NH–$	ND	>128	ND	ND	ND	ND	ND
27	8983	$R^1 = R^2 = CH_3(CH_2)_{10}C(NH)NH–$	ND	16	ND	ND	ND	ND	8
32	9026	$R^1 = R^2 = H_2NC(NH)NH–$	>128	>128	>128	ND	ND	ND	>128
EMB [c]			8	16–32	8	8	8–16	32	16
AMK [c]			0.5–2	1–2	0.5–2	1–2	0.5–1	2–4	1–2

[a] The minimum inhibitory concentration (MIC) was determined for *M. tuberculosis* (MTB H37Ra) and several *M. avium* (MAV) clinical isolates using either a macrodilution broth assay or a colorimetric microdilution broth assay.

[b] ND, not done.

[c] Ethambutol (EMB) and amikacin sulfate (AMK) were used as positive controls. MICs are given as the range of values obtained with multiple assays.



Scheme 2.

procedures for the conversion of **1** to **4** have already been referenced, experimental details have not been repeated, but some departures from the literature[29] in the sequence leading from **4** to **7** justify inclusion of compounds **5**–**7** in Section 3.

Starting material **7** was found to be poorly soluble in a variety of common reaction solvents, including pyridine and *N*,*N*-dimethylformamide. This problem was relieved somewhat by the synthesis of compound **8**, obtained easily by catalytic hydrogenation of diazide **5** in the presence of hydrochloric acid to protonate the amine as it formed. This modification was required to block the well-known oxygen-to-nitrogen benzoyl migration. A similar acyl migration in the trehalose system has been reported.[31] The problem of benzoyl migration in precursor **8** could have been avoided by use of the corresponding hexa-*O*-benzyl compound,[31] however, we employed intermediates **4** and **8** as precursors in early experiments simply because they were on hand. In a case where migration was allowed to proceed in order to obtain a sample of **9** for characterization, it was observed that in CH_2Cl_2 the process was fairly slow at room temperature, with several days being required for completion. Also, the migration is not a process of random benzoate ester aminolysis, since ^{1}H NMR spectral analysis showed that a single species, structure **9**, was present. Compound **8** could be stored in a freezer under dry conditions for several weeks. A far more significant limitation of **7** as a starting material was the unexpectedly weak nucleophilicity of its amino groups, a characteristic shared by its benzoylated derivative **8**. In fact, of the several classes of target compounds mentioned above, only sulfonyl chlorides (Scheme 2) were sufficiently reactive with **8** to generate the desired products in acceptable yields. Attempted aminolysis of a series of methyl imidate esters (**23**, Scheme 2) by **7** gave poor results. However, it is noteworthy that reaction of **7** with a masked guanidino precursor (Fig. 3) with elimination of methanethiol went slowly in *N*,*N*-dimethylformamide.

Sulfonamides.—The first group of potential antimycobacterial agents are the sulfonamido compounds shown in Scheme 2. The free base form of **8** was generated in situ from **8**·**2**HCl with $NaHCO_3$. Reaction of **8** with four commercially available sulfonyl chlorides (butyl, octyl, hexadecyl, phenyl) in CH_2Cl_2 solution in the presence of excess Et_3N gave intermediates **10a**–**d** in moderate to good yields as brittle foams. Although the migration product **9** was present in the crude reaction mixtures, it was easily removed by silica gel column chromatography. Conventional deprotection with NaOMe–MeOH gave target compounds **11a**–**d** as crystalline solids.

N-*Alkylamines.*—Several different approaches were used to prepare a series of *N*-alkylated derivatives of diaminotrehalose (Fig. 1). An attempt to directly alkylate **7** with dodecyl iodide in dilute *N*,*N*-dimethylacetamide (DMAc) solution at room temperature showed insignificant product formation after 3 days. Increasing the temperature to 50 °C gave a complex mixture of *N*-alkylated, mostly quaternized, products. None of the desired 6,6′-bis(monoalkylamine) could be isolated, but a few milligrams of the 6,6′-bis(dialkylamino) compound **12** was obtained by column chromatography for bioassay. Repetition of this reaction at room temperature using the benzoyl-protected compound **8** as starting material showed the same unreactivity. After 3 days, an 80% yield of benzoyl migration product **9** was recovered. An attempt to condense both amino groups of **8** with dodecyl aldehyde, followed by reduction of the expected intermediate **13** (Fig. 1) to the desired 6,6′-didodecyl compound was unsuccessful. Mass-spectral analysis showed several unidentified components of high mass (polymeric material?), but none of the expected **13**.

An alternative and more successful approach involving direct displacement of the 6,6′-dibromotrehalose with alkylamines was used for the preparation of compounds **15 a**–**d** (Fig. 1). Reaction of the 6,6′-dibromotrehalose **4** with *n*-octylamine and with *n*-dodecylamine gave intermediates **14a** and **14b**, and prolonged reaction with these primary amines removed the benzoyl groups as well, thus generating target compounds **15a** and **15b** in a single step. However, preparation of intermediates **14c** and **14d** by reaction of **4** with secondary amines required activation by NaI and high reaction temperatures, followed by conventional deprotection with NaOMe–MeOH or NH_3–MeOH to generate **15c** and **15d**.

It became obvious that a different and more reactive intermediate than either **4**, **7**, or **8** was needed. The benzoyl-protected 6,6′-ditriflate **16** (Fig. 1*)* seemed attractive, as the acetyl-,[32] benzyl-,[32] and benzoyl-

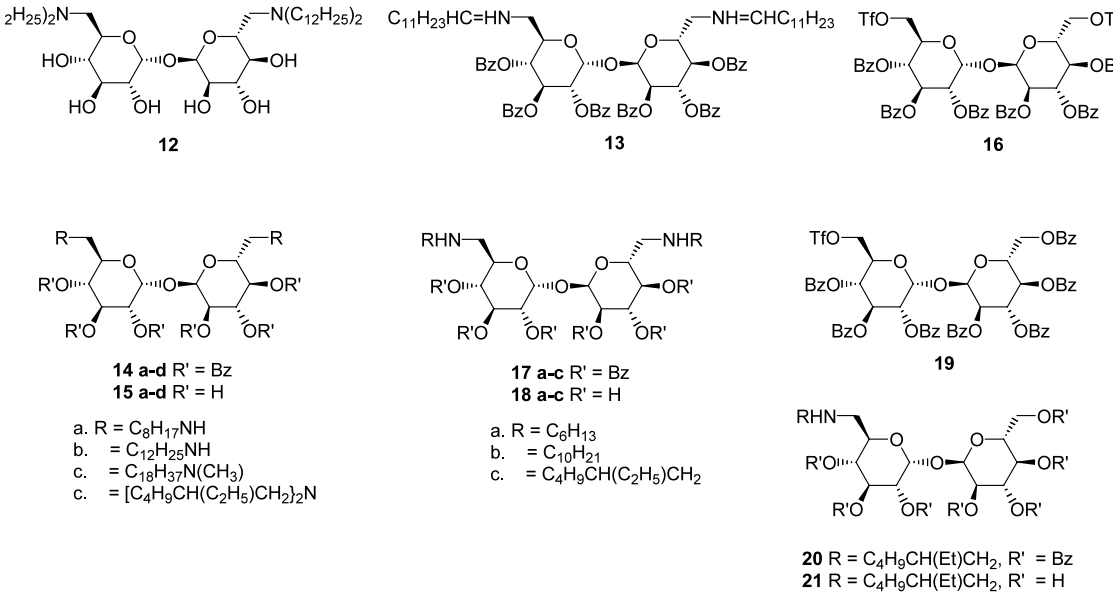


Fig. 1. *N*-Alkylated derivatives of diaminotrehalose.

protected[33] ditriflates have been reported. In addition to these literature procedures, we had published[34] complete experimental details for conversion of anhydrous trehalose to **16** in four steps. As expected, the reactions of **16** with alkylamines to give compounds **17a**–**c** were faster and cleaner than previous methods, and in retrospect, all of the alkylamines reported in Fig. 1 could have been prepared most easily from **16**. An unexpected result was encountered in the cases where conventional NaOMe in MeOH debenzoylation was employed to obtain the target alkylamines **14 a**–**d** and **17 a**–**c**. All benzoyl-protected starting materials (**14c**,**d**; **17b**,**c**) were consumed within 1 h to give the expected product as an iodine-absorbing, but non-UV-active (fluorescence quenching) spot on TLC, accompanied by a slightly faster-moving, UV-active spot that was not significantly affected by increasing reaction time to 3 h. However, exposure of crude **18b** to liquid NH_3 for 24 h diminished the byproduct spot and increased the desired product spot significantly. This extraordinary base stability plus MS evidence that showed **18b** with *one* remaining Bz group suggested that the byproduct was an *N*-alkyl-*N*-benzoyl compound. This was confirmed in the preparation of **18c** by isolation of the byproduct. MS showed **18c** with one extra Bz group (*m*/*z* 669), and IR showed no benzoyl ester (C=O) absorption, but was consistent with a substituted benzamide. Careful reinspection of the starting material **17c** (NMR) showed that O to N benzoyl migration had not already occurred; thus, the *N*-benzoyl byproduct arose during the deblocking procedure. All of these crude deprotection products could have been reworked via the liquid NH_3 method to maximize yields, but our main emphasis was simply to obtain an adequate quantity of pure target compounds for biological assay.

We had reported previously (Ref. 34; page 175) that 2,3,4,2′,3′,4′,6′-hepta-*O*-benzoyltrehalose had been isolated as a byproduct in the synthesis leading to **16**. Reaction of this compound with triflic anhydride gave the monotriflate **19**. Displacement with 2-ethylhexylamine followed by debenzoylation gave target compound **21** (Fig. 1) in which only one glucopyranose ring bears an alkylamine substituent.

Amidines.—Another class of potential antimycobacterial agent that received considerable effort but limited success is illustrated by compounds like **24** shown in Fig. 2. Following a literature procedure,[35] a series of methyl imidate esters **23a**–**d** (Fig. 2) were prepared by the dry HCl-catalyzed addition of anhydrous MeOH to the cyano group of four commercially available nitriles, [RCN **22a**–**d**, where $R = C_5H_{11}$ (**22a**), $C_{11}H_{23}$ (**22b**), $C_6H_5CH_2$ (**22c**), (2-naphthyl)CH_2 (**22d**)]. The products **23a**–**d** were obtained as solid hydrochloride salts with limited solubility in cold Et_2O, which was used to wash out two byproducts (namely the corresponding amide, $RCONH_2$, and the ester, RCO_2Me). Except for an NMR spectrum for **23d**, which was obtained to confirm

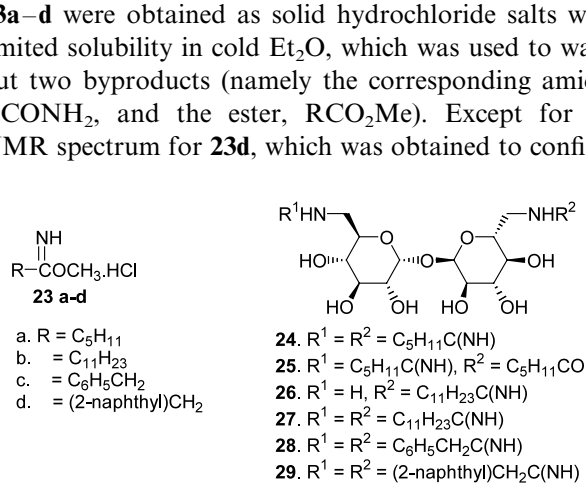


Fig. 2. Amidine derivatives of trehalose.

its structure, these intermediates were used without characterization. Mass-spectral data, however, obtained on subsequent reaction products known to contain the appropriate unreacted **23** provided additional confirmation. Reaction of 6,6′-diamino-6,6′-dideoxytrehalose (**7**) with an excess of each of these imidate esters (free base) was expected to effect aminolysis of the esters to generate trehalose derivatives substituted at the 6-positions by imidoamide (amidine) groups to give target structures **24**, **27**, **28**, and **29**. Unfortunately, the weak nucleophilicity of the amino groups in **7** was very evident here. For the two aliphatic esters (**23a** and **23b**), formation of the mono(amidine) by reaction at only one 6-position could be detected by TLC after a few hours, increasing to perhaps 25–30% after a week or more. There was even less of the 6,6′-bis(amidine), probably no more than 10–15% of the reaction product as estimated by TLC. For the two esters with aromatic substituents (**23c** and **23d**), no amidine products (**28** or **29** or their mono(amidine) analogs) were ever isolated in sufficient quantity and purity to justify analysis and bioassay. A solution of **7** and **23d** in methanol, where R = (2-naphthyl)CH_2– was also refluxed under N_2 for 20 h, but no product **29** was obtained. In a parallel series of reactions of methyl-5-amino-5-deoxy-D-arabinopyranoside with five imidate esters [**23a**–**d** plus a fifth with R = $CH_3(CH_2)_{14}$–], only reactions with **23b** and **23d** gave sufficient arabinose-5-(amidine) for bioassay (Dr Joyce Friedrich, unpublished results).

Besides sluggish reactivity, another troublesome aspect of the amidine chemistry was the nearly intractable purification problems. A typical reaction mixture contained large quantities of unreacted imidoester, along with its byproducts $RCONH_2$ and RCO_2CH_3. Trehalose components included unreacted diaminotrehalose, the 6-mono(amidine), the 6,6′-bis(amidine), and their corresponding normal amides formed either by degradation of the amidines or by aminolysis of the minor ester contaminant present in the starting material **23**. Chromatographic resolution of this latter group was hindered by close spacing of bands and by their highly polar nature, which led to severe trailing on the column. No neutral, polar solvent, or solvent mixture was found that produced adequate mobility and resolution on either silica gel or cellulose, but the best compromise was found to be mixtures containing both alcohols and acetic acid, such as *n*-BuOH–HOAc–H_2O or $CHCl_3$–MeOH–HOAc, used with coarse silica gel (70–230 mesh) contained in a flash column pressurized with N_2 to provide an adequate flow rate. The presence of the acetic acid sharpened the resolution considerably while at the same time leaching silica from the column packing and accelerating the otherwise slow degradation of amidine to normal amide. In a single trial purification with styrene–divinylbenzene copolymer beads (Bio-Beads™ SM-4), a complex mixture of trehalose components applied in water solution was readily adsorbed, but a very large volume of eluant containing 50% MeOH was required to elute the organic components, along with a large quantity of plastic material leached from the column. The net effect was to substitute one group of contaminants for another.

Because of these problems, only compounds **24**, **26,** and **27** were characterized sufficiently to justify biological evaluation. Compound **24** appeared to be of high purity by TLC and MS, but the sample was submitted for biological evaluation as a mixture of ~90% **24** and ~10% **25**, as their acetic acid salts, based on elemental analysis and NMR data. Likewise, the mono(amidine) **26** and the corresponding bis(amidine) **27** gave satisfactory mass and ^1H NMR spectra, but were contaminated with silica. However, compound **27** showed significant antimycobacterial activity.

Diguanidino.—The 6,6′-diguanidino compound **32** (Fig. 3) was obtained by a route that avoided difficult purification problems like those associated with the similar amidines. *N*,*N*′-Bis(benzyloxycarbonyl)-*S*-methylisothiourea (**30**) (Fig. 3) was obtained by reacting *S*-methylisothiourea with benzyl chloroformate according to a literature procedure.[36] Reaction of **30** with 6,6′-diamino-6,6′-dideoxytrehalose (**7**) in DMF for 6 days gave intermediate **31** as a mixture with ~10% of a similar product with three Cbz groups instead of four as shown by MS: 827 $[M+H]^+$. Deprotection of the mixture **31** by catalytic hydrogenolysis in MeOH with 10% Pd-on-charcoal in the presence of a little $CHCl_3$ to serve as an in situ source of HCl[31] gave the 6,6′-diguanidino compound **32** as its dihydrochloride salt with no need for further purification.

Antimycobacterial activity.—The compounds listed in Table 1 were evaluated for antimycobacterial activity against *M. tuberculosis* strain $H_{37}Ra$ and a panel of three to five clinical isolates of *M. avium*. Minimum inhibitory concentrations (MIC) for each compound were determined using either a colorimetric microdilution broth assay (for a detailed description of the procedures, see Ref. 38) or a macrodiltuion broth assay. For ethambutol and amikacin, used against the same strains as controls, MIC's ranged from 8 to 32 μg/mL and 0.5 to 4 μg/mL, respectively.

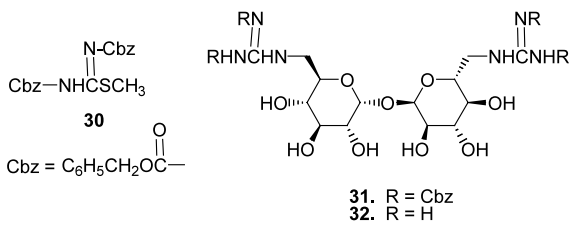


Fig. 3. 6,6′-Diguanidino trehalose derivatives.

6,6′-Diamino-6,6′-dideoxytrehalose (**7**) and its diazido precursor **6** were both inactive, but significant activity apparently related to aliphatic chain length was found among the sulfonamides, *N*-alkylamines, and one of the amidines. Many more examples of each class would be required to fully elucidate structure–activity relationships, but among the compounds tested, the following trends were noted.

- The C_8-alkylsulfonamido compound **11b** was the most active compound of this group; the C_{16}, C_4 and phenyl analogs were inactive at the highest concentrations tested.
- Among the *N*-alkylamines, the C_8, C_{10}, and C_{12} compounds **15a**, **18b**, and **15b** showed consistently good activity against the mycobacterial panel; the C_6 analog **18a** and the branched-C_8 (2-ethylhexyl) compound **18c** showed a broader range of activities against different strains of mycobacteria. 6,6′-Bis(tertiaryamino) compounds **12** and **15d** were less active than their bis(secondary-amino) counterparts **15b** and **18c**. Tertiary amine **15c** with a C_{18} substituent was also inactive. It was interesting that compound **21**, substituted in only *one* glucopyranose ring with the (2-ethylhexyl)amino group, was inactive, but the 6,6′-disubstituted counterpart **18c** was moderately active.
- The C_{12} bis(amidine) **27** showed good activity against the two *M. avium* strains tested, but again, the monosubstituted counterpart **26** was inactive for the single strain tested. The C_6 bis(amidine) **24** was inactive.
- The 6,6′-diguanidino compound **32** was inactive.
- In addition to the compounds in Table 1, the benzoyl-protected precursors **10b**, **10c**, **10d**, **17a**, and **17b** were also screened; all were inactive.

3. Experimental

General methods.—Melting points were determined by the capillary method on a Mel-Temp apparatus and are uncorrected. Reaction temperatures below the boiling point of the indicated solvent were external oil bath temperatures. Evaporations were carried out in vacuo on a rotary evaporator or under high vacuum by short-path distillation into a glass trap cooled in dry ice–acetone. Samples were dried under high vacuum over P_2O_5 at rt unless an elevated temperature is specified. Many of the alkylamines reported herein were obtained as soft, waxy, but tractable, foams with low, indistinct melting points by flash evaporation from concentrated solution in CH_2Cl_2. Those with the longer alkyl chains exhibited detergent behavior in water–organic solvent mixtures, and some attempts at recrystallization from alcohol solvents resulted in the formation of gels. Elemental analyses were performed by the Molecular Spectroscopy Section of the Southern Research Institute or by Atlantic Microlab, Inc., Norcross, GA. Where solvents are included in the reported analysis, their presence was confirmed by the ^{1}H NMR spectrum. Analtech precoated (250 μm) Silica Gel G (F) plates were used for thin-layer chromatography (TLC), and spots were detected by irradiation with a UV-254 light, by absorption of iodine vapor, or by charring after a $(NH_4)_2SO_4$–H_2SO_4 spray. Column chromatography in either the flash column or gravity mode was performed with 230–400 mesh silica gel (SG) using the slurry method of column packing, except in a few cases where the coarser 70–230 mesh is specified. Mass spectra were recorded on a Varian/MAT 311A double-focusing mass spectrometer in the fast-atom bombardment (FAB) mode. Addition of a trace of LiCl to some samples gave an $[M + Li]^+$ cluster peak that was stronger than the $[M + H]^+$ peak. For many compounds a particularly prominent mass peak was generated by rupture on both sides of the glycosidic bond to give *two* glucopyranosyl units. In the mass spectral data for each compound the symbol $1/2[M - 16]^+$ should be understood to represent the appropriate glucopyranosyl unit complete with its various substituent groups. Infrared spectra were recorded on a Nicolet FT IR spectrophotometer, model 10DX, using pressed KBr discs or capillary films. Peak positions are reported in wavenumbers (ν) in cm^{-1}. ^{1}H NMR spectra were recorded on a Nicolet/GE NT300NB spectrometer operating at 300.635 MHz. Chemical shifts (δ) are reported in ppm downfield from internal Me_4Si. Chemical shifts of multiplets are measured from the approximate center of the multiplet, and coupling constants (J) are reported in Hz. Because of the remarkable symmetry of the trehalose molecule, equivalent protons in both rings have the same chemical shift, but it should be noted that the CH_2 protons located at the 6-position in both rings are nonequivalent, and they may couple with other protons with only slightly different J values, giving rise to *apparent* triplets or quartets on first-order inspection which are, in fact, two doublets. In the ^{1}H NMR data for the reported compounds of Scheme 1 and for the complete set of protected and deprotected compounds of Scheme 2, we have identified and documented the relevant couplings. However, inspection shows little variation in coupling patterns and J values within a structural group; therefore, the other schemes include detailed coupling data for a representative compound of that group. Similar data for other compounds are available upon request. UV spectra were not obtained for all samples because of the lack of significant absorption above 200 nm for many of the deprotected target compounds, but for selected samples they were determined in 0.1 N HCl (pH 1), pH 7 buffer, and 0.1 N NaOH (pH 13) with a Perkin–Elmer UV–Vis-near infrared model Lambda 9 spectrophotometer. Maxima

are reported in the format: λ_{max} in nanometers ($\varepsilon \times 10^{-3}$).

6,6′-Diazido-2,3,4,2′,3′,4′-hexa-O-benzoyl-6,6′-dideoxy-α,α-trehalose (**5**).—Intermediates **2**–**4** (Scheme 1) had been prepared from anhyd α,α-trehalose (**1**) by known procedures.[26–30] Under an N_2 atmosphere, a stirred mixture of **4** (91.5 g, 83.7 mmol) and excess NaN_3 (71.4 g, 1.1 mol) in dry DMF (1 L) was heated for 14 h at 65 °C. After cooling, the dark mixture was poured with vigorous mechanical stirring into 15 L of ice and water. The precipitate was collected by filtration, washed with cold water and cold isopropyl alcohol, and then recrystallized from 5 L of boiling isopropyl alcohol with filtration through a preheated Buchner funnel. The dense crystalline deposit was collected, washed with cold 2-PrOH, and dried to constant weight at rt; yield 76.5 g (90%); mp 184–185 °C. A 200-mg portion was recrystallized from 2-PrOH (40 mL) to give an analytical sample. The long white needles were dried at rt overnight and then at 65 °C for 1 h; yield 187 mg; mp 185–186 °C (lit.[29] 180–182 °C). MS: (with LiCl) m/z 1023 $[M + Li]^+$, 500 $(1/2[M - 16])^+$. IR: 2107 cm^{-1} (azido), 1730 cm^{-1} (ester C=O). ^1H NMR (Me_2SO-d_6): δ 3.27 (d, 4 H, J 4.1 Hz, 6-CH_2), 4.02 (m, 2 H, $J_{4,5}$ 9.9, $J_{5,6} = J_{5,6'} = 4.1$ Hz, H-5), 5.59 (dd, 2 H, $J_{3,4}$ 9.6 Hz, H-4), 5.67 (dd, 2 H, $J_{1,2}$ 3.5, $J_{2,3}$ 10.0 Hz, H-2), 5.77 (d, 2 H, H-1), 6.10 (app. t, 2 H, H-3), 7.41–7.69, 7.83, 8.06 (complex m, 30 H, Bz). Anal. Calcd for $C_{54}H_{44}N_6O_{15}$: C, 63.78; H, 4.36; N, 8.26. Found: C, 63.60; H, 4.28; N, 8.05.

6,6′-Diazido-6,6′-dideoxy-α,α-trehalose (**6**).—Solid NaOMe (1.59 g, 30.0 mmol) was added to a suspension of **5** (30.0 g, 29.5 mmol) in dry MeOH (1500 mL), and the mixture was stirred at rt for 48 h, having become a clear solution after ~20 h. The solution was neutralized by portionwise addition of Dowex 50W-X8 (H^+ form) cation exchange resin. The resin was filtered off, and evaporation of the filtrate gave a semisolid residue that was washed by trituration and decantation with hexane (3 × 50 mL) to remove methyl benzoate. A solution of the residual solid in hot isopropyl alcohol (700 mL) was treated with charcoal (5 g) and Celite (20 g) and filtered through a thin Celite pad to remove a colloidal turbidity assumed to be NaCl. On cooling, the clear filtrate deposited dense white crystals, which were collected, washed with 2-PrOH, and dried, first at rt and then at 82 °C for 18 h; yield 8.41 g (73%); mp 211–212 °C (dec with gas evolution); lit.[29] mp 195–198 °C. Concentration of the filtrate to ~100 mL gave a second crop of lower melting material; 3.0 g (26%); mp 197–200 °C. Recrystallization of a 200 mg portion of the first crop from 2-PrOH (30 mL) to give a sample for analysis did not change the melting point; yield 151 mg. MS: m/z 393 $[M + H]^+$. IR: 2106 cm^{-1} (azido). ^1H NMR (Me_2SO-d_6): δ 3.10 (complex m, 2 H, H-4), 3.30 (complex m, 2 H, H-2), 3.39 (d, 4 H, J 4.0 Hz, 6-CH_2), 3.55 (m, 2 H, H-3), 3.96 (m, 2 H, H-5), 4.91 (m, 6 H, H-1, OH-2, and OH-3 overlap), 5.13 (d, 2 H, J 4.8 Hz OH-4). A trace of 2-PrOH was noted. Anal. Calcd for $C_{12}H_{20}N_6O_9 \cdot 0.1\ C_3H_8O$: C, 37.09; H, 5.26; N, 21.10. Found: C, 37.05; H, 5.15; N, 21.15.

6,6′-Diamino-6,6′-dideoxy-α,α-trehalose (**7**).—A solution of **6** (8.05 g, 20.53 mmol) in CH_3OH (900 mL) was hydrogenated at atmospheric pressure by stirring with 5% Pd–C catalyst (1 g) for 27 h. The mixture was filtered through a Celite pad under N_2 pressure, and the catalyst was washed with 2 × 100 mL of hot CH_3OH. Evaporation of the filtrate gave a white solid, 2.93 g (42%), which was purified by extraction into CH_3OH (150 mL) in a Soxhlet apparatus. The solid that deposited in the cooled extract was dried at 82 °C for 18 h to give a sample that analyzed as the 0.6 MeOH solvate. After redrying overnight at 82 °C and 2 h at 100 °C, the composition was virtually unchanged; yield 1.87 g (25%); mp sinters and chars gradually above ~218 °C. MS: m/z 341 $[M + H]^+$, 162 $(1/2[M - 16]^+)$. ^1H NMR (Me_2SO-d_6): δ 2.56, 2.60 (2d, 2 H, $J_{6,6'}$ 13.3, $J_{5,6'}$ 6.4 Hz, 6-CH_2), 2.75, 2.80 (2d, 2 H, $J_{5,6}$ 3.3 Hz, 6-CH_2), 3.06 (app. t, 2 H, $J_{3,4}$ 8.5, $J_{4,5}$ 8.8 Hz, H-4), 3.26 (dd, 2 H, $J_{1,2}$ 3.5, $J_{2,3}$ 9.6 Hz, H-2), 3.54 (app. t, 2 H, H-3), 3.61 (m, 2 H, H-5), 0.8–2.1 and 4.0–5.8 (very broad, NH, OH, best seen in integral). MeOH was noted. Anal. Calcd for $C_{12}H_{24}N_2O_9 \cdot 0.6\ CH_3OH$: C, 42.09; H, 7.40; N, 7.79. Found: C, 42.33; H, 7.33; N, 7.78.

6,6′-Diamino-2,3,4,2′,3′,4′-hexa-O-benzoyl-6,6′-dideoxy-α,α-trehalose dihydrochloride (**8**).—A suspension of **5** (5.00 g, 4.92 mmol) in EtOH (1 L) and 1.0 N HCl (11.0 mL) was hydrogenated at atmospheric pressure by stirring with 5% Pd–C catalyst for 28 h. After filtration of the mixture through a thin Celite pad and liberal washing of the pad with EtOH, the filtrate was evaporated using a lukewarm water bath. The residue was purified by reprecipitating twice from MeOH (50 mL) solution by dropwise addition of Et_2O (250 mL). The solid was collected by filtration under N_2 pressure, washed with Et_2O, and dried at rt overnight and at 65 °C for 3 h; yield 3.14 g (57%). MS: m/z 965 $[M + H]^+$. ^1H NMR (Me_2SO-d_6): δ 2.96, 3.02 (2d, 2 H, $J_{5,6}$ 7.0, $J_{6,6'}$ 13.6 Hz, 6-CH_2), 3.08, 3.12 (2d, poorly resolved, 2 H, $J_{5,6'}$ 2.4 Hz, 6-CH_2), 4.20 (m, 2 H, $J_{4,5}$ 10.1, $J_{5,6'}$ 2.4 Hz), 5.76 (app. t, 2 H, $J_{3,4}$ 9.5 Hz, H-4), 5.92, 5.96 (m, 4 H, $J_{1,2}$ 3.5, $J_{2,3}$ 9.7 Hz, H-1 and H-2), 6.27 (app. t, 2 H, H-3), 7.36–7.80, 8.13 (complex m, 30 H, Bz), 8.26 (very br, $NH_2 + H^+$). Very large water peak noted. Anal. Calcd for $C_{54}H_{48}N_2O_{15} \cdot 2\ HCl \cdot 4.3\ H_2O$: C, 58.15; H, 5.30; N, 2.51. Found: C, 58.18; H, 5.00, N, 2.73.

2,3,2′,3′-Tetra-O-benzoyl-6,6′-bis(benzoylamino)-6,6′-dideoxy-α,α-trehalose (**9**).—A byproduct believed to be compound **9** had been observed to form slowly at rt in reaction mixtures involving **8** in neutral or basic media.

An authentic sample of **9** was prepared for positive identification by modifying the procedure for preparation of **8**. The procedure for preparation of **8** from **5** was repeated exactly except that no HCl was added, and the reaction flask was warmed for 5 h in a 50 °C water bath during the hydrogenation. After 24 h, filtration and evaporation of the filtrate gave 4.7 g of crude product. Column chromatography on silica gel with 97:3 $CHCl_3$–MeOH as eluant gave three main components. The first was a stable white solid (2.44 g, 51%) that was shown by spectral data to be **9**. The analytical sample was obtained by two recrystallizations from EtOH followed by drying for 6 h at 65 °C; mp 201–202 °C. MS: m/z 965 $[M+H]^+$, 474 $(1/2[M-16]^+)$, 105 $[Bz]^+$. IR: 1731 cm^{-1} (ester C=O), 1637 cm^{-1} (amide). ^1H NMR (Me_2SO-d_6): δ 3.03, 3.21 (2m, 4 H, $J_{6,NH}$ 4.6, $J_{5,6}$ 2.9, $J_{6,6'}$ 14.3, $J_{6',NH}$ 6.1, $J_{5,6'}$ 4.3 Hz, 6-CH_2), 3.76 (m, 2 H, $J_{4,4\text{-OH}}$ 5.0, $J_{3,4}$ 9.2, $J_{4,5}$ 10.0 Hz, H-4), 3.85 (m, 2 H, H-5), 5.28 (dd, 2 H, $J_{1,2}$ 3.7, $J_{2,3}$ 10.3 Hz, H-2), 5.51 (d, 2 H, H-1), 5.67 (d, 2 H, 4-OH), 5.79 (app. t, 2 H, H-3), 7.47, 7.51, 7.78, 7.95 (complex m, 30 H, Bz), 8.18 (t, 2H, BzN*H*–). Strong water peak noted. Anal. Calcd for $C_{54}H_{48}N_2O_{15}\cdot 0.9\ H_2O$: C, 66.10; H, 5.12; N, 2.86. Found: C, 66.12; H, 5.13; N, 2.89.

The other identified products obtained from the column were **8** ($\sim$3%) and the 6-benzamido-6′-amino compound (1.48 g, 31%) formed by O^4 to N^6 Bz migration in *one* ring, but not both. Spectral analysis of this transient intermediate shortly after it was obtained from the column gave a FAB mass spectrum identical to **9**, as expected, but the ^1H NMR was more complicated because of the unsymmetrical molecule. ^1H NMR (Me_2SO-d_6): δ 2.14, 2.31 (2m, 2 H, 6′-H_2NCH_2–), $\sim$3.32 (m, water superimposed on 6-BzNHCH_2–), 3.82 (complex m, 3 H, H-4, H-5, H-5′), 5.33 (dd, 1 H, $J_{1,2}$ 3.7, $J_{2,3}$ 10.5 Hz, H-2), 5.51 (m, 1 H, H-2′), 5.54 (d, 1 H, H-4′), 5.60 (d, 1 H, H-1), 5.66 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1′), 5.74 (d, 1 H, $J_{4,4\text{-OH}}$ 4.8 Hz, 4-OH), 5.87 (app. t, 1 H, H-3), 6.05 (app. t, 1 H, H-3′), 7.36–8.08 (complex m, 30 H, Bz), 8.27 (t, 1 H, 6-BzN*H*CH_2). On a TLC plate with (97:3) $CHCl_3$–MeOH as solvent, these components had the following R_f values: **5** (0.93), **9** (0.91), 6-benzamido-6′-amino intermediate (0.55), **8** (0.13).

2,3,4,2′,3′,4′-Hexa-O-*benzoyl-6,6′-bis[(1-butylsulfonyl)amino]-6,6′-dideoxy-α,α-trehalose* (**10a**).—Solid $NaHCO_3$ (97 mg, 1.16 mmol) was added to a solution of the amine dihydrochloride **8** (600 mg, 0.58 mmol) in MeOH (10 mL). After stirring for 5 min, CH_2Cl_2 (50 mL) was added, and the mixture was evaporated at rt. Dry CH_2Cl_2 (2 × 25 mL) was added and evaporated to aid complete removal of MeOH. A solution of the residue in dry CH_2Cl_2 (25 mL) was treated with Et_3N (304 mg, 3.0 mmol) followed by 1-butanesulfonyl chloride (219 mg 1.40 mmol) and stirred at rt under N_2 for 3 days. Volatiles were evaporated; a solution of the residue in $CHCl_3$ was washed by shaking with water, satd $NaHCO_3$, and water. Evaporation of the dried (Na_2SO_4) organic layer gave 0.75 g of yellow foam. Purification of the crude product by chromatography on silica gel with (99:1) $CHCl_3$–MeOH as eluant gave **10a** as a soft, but tractable, glassy white foam; yield 529 mg (76%); mp softens to a syrup gradually above $\sim$92 °C. MS: m/z 1207 $[M+H]^+$. IR: 1735cm^{-1} (ester C=O), 1330, 1317, 1144 (SO_2NH). UV (EtOH): 0.1 N HCl 240 (66.5), 276 (sh), 285 (sh); pH 7 buffer 241 (64.1), 276 (sh), 285 (sh); 0.1 N NaOH 233 (67.7), 276 (9.2), 284 (sh). ^1H NMR ($CDCl_3$): δ 0.98 (t, 6 H, CH_3), 1.47 (m, 4 H, CH_3CH_2–), 1.78 (m, 4 H, $CH_3CH_2CH_2$–), 2.59, 2.90 (2m, 4 H, $J_{5,6}$ 3.8, $J_{6,6'}$ 14.7, $J_{6,NH}$ 6.4, $J_{5,6'}$ 2.6, $J_{6',NH}$ 7.1 Hz, 6-CH_2), 2.97 (m, 4 H, CH_2-SO_2NH), 3.93 (2m, 2 H, $J_{4,5}$ 9.3, $J_{5,6}$ 2.6, $J_{5,6'}$ 3.8 Hz, H-5), 4.70 (app. t, 2 H, SO_2NH), 5.33 (dd, 2 H, $J_{1,2}$ 3.9, $J_{2,3}$ 10.3 Hz, H-2), 5.40 (app. t, 2 H, $J_{3,4}$ 9.5 Hz, H-4), 5.70 (d, 2 H, H-1), 6.25 (app. t, 2 H, $J_{3,4}$ 9.5 Hz, H-3), 7.33, 7.43, 7.59, 7.82, 7.91, 8.07 (complex m, 30 H, Bz). Anal. Calcd for $C_{62}H_{64}N_2O_{19}S_2$: C, 61.78; H, 5.35; N, 2.32. Found: C, 61.50; H, 5.51; N, 2.47.

2,3,4,2′,3′,4′-Hexa-O-*benzoyl-6,6′-dideoxy-6,6′-bis-[(1-octylsulfonyl)amino]-α,α-trehalose* (**10b**).—Using the following quantities of reagents, **10b** was obtained by the same method given in detail for **10a** above: **8** (717 mg, 0.74 mmol), $NaHCO_3$ (125 mg, 1.49 mmol), MeOH (10 mL), Et_3N (202 mg, 2.0 mmol), 1-octanesulfonyl chloride (319 mg, 1.50 mmol), CH_2Cl_2 for reaction solvent (20 mL); reaction time was 48 h. Column chromatography on silica gel with (99:1) $CHCl_3$–MeOH as eluant gave **10b** as a white glassy foam; yield 536 mg (55%); mp softens to a syrup above $\sim$70 °C. MS: m/z (with LiCl) 1323 $[M+Li]^+$, 650 $(1/2[M-16]^+)$, 528 $[650-PhCOOH]^+$, 406 $[528-PhCOOH]^+$. UV (EtOH; turbid at pH 1 and 7): 0.1 N HCl 242, 276 (sh), 284 (sh); pH 7 buffer 242, 276 (sh), 284 (sh); 0.1 N NaOH 232 (69.5), 275 (7.4), 284 (sh). ^1H NMR ($CDCl_3$): δ 0.89 (t, 6 H, CH_3), 1.31, 1.42, 1.78, 2.95 (4m, 16 H, 4 H, 4 H, 4 H, aliphatic CH_2), 2.60, 2.88 (2m, 4 H, 6-CH_2), 3.93 (2m, 2 H, $J_{4,5}$ 10.0 Hz, H-5), 4.68 (t, 2 H, SO_2NH), 5.31, 5.34 (dd, 2 H, $J_{1,2}$ 3.9, $J_{2,3}$ 10.3 Hz, H-2), 5.39 (app. t, 2 H, J 9.6, J 9.7 Hz, H-4), 5.71 (d, 2 H, H-1), 6.24 (app. t, 2 H, H-3), 7.32, 7.43, 7.58, 7.83, 7.91, 8.07 (complex m, 30 H, Bz). Anal. Calcd for $C_{70}H_{80}N_2O_{19}S_2$: C, 63.81; H, 6.12; N, 2.13. Found: C, 63.91; H, 6.18; N, 2.33.

2,3,4,2′,3′,4′-Hexa-O-*benzoyl-6,6′-dideoxy-6,6′-bis-[(1-hexadecylsulfonyl)amino]-α,α-trehalose* (**10c**).—The crude product obtained by reaction of **8** (500 mg, 0.52 mmol) and 1-hexadecanesulfonyl chloride (390 mg, 1.2 mmol) by the method of **10a**, above, was chromatographed on silica gel with (99:1) $CHCl_3$–MeOH as eluant to give **10c** (545 mg, 68%) as a glassy foam. A second column on a 360 mg portion of this to remove a trace impurity followed by drying for 24 h gave the analytical sample; 201 mg; mp softens to a syrup above

~45 °C. MS (with LiCl): *m/z* 1547 $[M+Li]^+$. UV (EtOH, turbid at pH 1 and 7): 0.1 N HCl 246, 277 (sh), 285 (sh); pH 7 buffer 244, 277 (sh), 285 (sh); 0.1 N NaOH 232 (69.2), 275 (8.15), 284 (sh). ^{1}H NMR ($CDCl_3$): δ 0. 88 (t, 6 H, CH_3), 1.24, 1.42, 1.78, 2.96 (4m, 48 H, 4 H, 4 H, 4 H, aliphatic CH_2), 2.60, 2.88 (2m, 4 H, $J_{5,6}$ 3.5, $J_{6,6'}$ 14.4, $J_{6,NH}$ 6.5, $J_{5,6'}$ 2.5, $J_{6',NH}$ 6.5 Hz, 6-CH_2), 3.93 (2m, 2 H, $J_{4,5}$ 10.0 Hz, H-5), 4.69 (app. t, 2 H, SO_2NH), 5.32, 5.35 (2d, 2 H, $J_{1,2}$ 3.8, $J_{2,3}$ 10.2 Hz, H-2), 5.40 (app. t, 2 H, H-4, $J_{3,4}$ 9.8, $J_{4,5}$ 10.0 Hz, H-4), 5.71 (d, 2 H, H-1), 6.25 (app. t, 2 H, H-3), 7.32, 7.43, 7.59, 7.84, 7.92, 8.08 (complex m, 30 H, Bz). Anal. Calcd for $C_{86}H_{112}N_2O_{19}S_2$: C, 66.99; H, 7.32; N, 1.82; S, 4.16. Found: C, 67.23; H, 7.40; N, 1.75; S, 4.02.

2,3,4,2′,3′,4′-Hexa-O-benzoyl-6,6′-dideoxy-6,6′-bis[(phenylsulfonyl)amino]-α,α-trehalose (**10d**).—Compound **8** (1.00 g, 1.05 mmol) was reacted with benzenesulfonyl chloride (389 mg, 1.05 mmol) by the procedure given for **10a**. Column chromatography on silica gel with $CHCl_3$ as eluant followed by drying for 20 h gave **10d** as a brittle white foam; yield 1.10 g (84%) mp softens gradually above ~110 °C. MS: *m/z* 1245 $[M+H]^+$, with LiCl 1251 $[M+Li]^+$. UV (EtOH, turbid at pH 1and 7): 0.1 N HCl 234, 273 (sh), 284 (sh); pH 7 buffer 234, 273 (sh), 284 (sh); 0.1 N NaOH 231 (69.7) 273 (8.46), 284 (sh). ^{1}H NMR ($CDCl_3$): δ 2.48, 2.75 (2m, 4 H, $J_{5,6}$ 4.5, $J_{6,6'}$ 14.4, $J_{6,NH}$ 6.1, $J_{5,6'}$ 2.5, $J_{6',NH}$ 7.3 Hz, 6-CH_2), 3.83 (2m, 2 H, H-5), 4.97 (app. t, 2 H, SO_2NH), 5.07 (dd, 2 H, $J_{1,2}$ 3.7, $J_{2,3}$ 10.3 Hz, H-2,), 5.26 (dd, 2 H, $J_{3,4}$ 9.5, $J_{4,5}$ 10.2 Hz, H-4), 5.31 (d, 2 H, H-1), 6.11 (app. t, 2 H, H-3), 7.4, 7.77, 7.88, 8.03 (complex m, 35 H, Bz and Ph). Anal. Calcd for $C_{66}H_{56}N_2O_{19}S_2$: C, 63.66; H, 4.53; N, 2.25. Found: C, 63.54; H, 4.50; N, 1.99.

6,6′-Bis[(1-butylsulfonyl)amino]-6,6′-dideoxy-α,α-trehalose (**11a**).—A solution of NaOMe in MeOH (0.21 mL of 1.74 N, 0.36 mmol) was added to a solution of **10a** (430 mg, 0.36 mmol) in dry MeOH (10 mL), and the solution was allowed to stand under N_2 for 1 h. The solution was diluted with an equal volume of water and adjusted to pH 5 by portionwise addition of Dowex 50W-X8 (H^+) cation exchange resin. The mixture was filtered, and the filtrate was evaporated to dryness. The residue was washed by trituration and filtration with Et_2O to extract methyl benzoate. The solid filter cake (198 mg) was recrystallized from isopropyl alcohol (20 mL); the white solid was collected by filtration, washed with 2-PrOH, and dried at 65 °C for 20 h; yield 163 mg (79%); mp 169–170 °C. MS: *m/z* 581 $[M+H]^+$, 563 $[M-OH]^+$, 282 ($1/2[M-16]^+$), 1161 $[2M+H]^+$. ^{1}H NMR (Me_2SO-d_6): δ 0.89 (t, 6 H, CH_3), 1.38, 1.64 (2m, 4 each, aliphatic CH_2), 3.03 (m, 8 H, CH_2SO_2NH overlapped by 6-CH_2), 3.28 (m, 2 H, $J_{1,2}$ 3.6, $J_{2,3}$ 9.8, $J_{2,2\text{-OH}}$ 5.7 Hz, H-2), 3.31 (m, 2 H, H-4 overlapped by water), 3.55 (m, 2 H, $J_{3,4}$ 10.0) Hz, H-3, 3.75 (m, 2 H, $J_{5,6}$ 2.2, $J_{5,6'}$ 6.5 Hz, H-5), 4.73 (d, 2 H, *J* 5.7 Hz, 2-OH), 4.88 (d, 2 H, *J* 4.8 Hz, 3-OH), 4.93 (app. d, 4 H, H-1 and 4-OH), 6.80 (app. t, 2 H, SO_2NH). Anal. Calcd for $C_{20}H_{40}N_2O_{13}S_2$: C, 41.37; H, 6.94; N, 4.82. Found: C, 41.16; H, 7.19; N, 4.62.

6,6′-Dideoxy-6,6′-bis-[(1-octylsulfonyl)amino]-α,α-trehalose (**11b**).—Compound **10b** (469 mg, 0.36 mmol) was debenzoylated by the procedure given in detail for **11a** The product was recrystallized from 2:1 water–EtOH (30 mL), collected, washed on the funnel with water and Et_2O, and dried at 82 °C for 20 h; yield 204 mg (82%), mp 187–188 °C with prior sintering. MS (with LiCl): *m/z* 699 $[M+Li]^+$. ^{1}H NMR (Me_2SO-d_6): δ 0.86 (t, 6 H, CH_3), 1.26, 1.34, 1.64 (3m, 24 H, aliphatic CH_2), 2.99 (complex m, 8 H, CH_2SO_2NH + H-4 + 6-C*H*), 3.26, 3.32 (m, 4 H, $J_{1,2}$ 3.6, $J_{2,2\text{-OH}}$ 5.8, $J_{2,3}$ 9.6 Hz, 6-C*H*′ + H-2), 3.55 (m, 2 H, $J_{3,3\text{-OH}}$ 4.8 Hz, H-3), 3.74 (m, 2 H, $J_{4,5}$ 9.8, $J_{5,6}$ 1.8, $J_{5,6'}$ 6.7 Hz, H-5), 4.70 (d, 2 H, $J_{2,2\text{-OH}}$ 5.8 Hz, 2-OH), 4.86 (d, 2 H, 3-OH), 4.92 (m, 4 H, $J_{4,4\text{-OH}}$ 5.2 Hz, H-1 + 4-OH), 6.77 (app. t, 2 H, SO_2NH). Anal. Calcd for $C_{28}H_{56}N_2O_{13}S_2{\cdot}0.5\ H_2O$: C, 47.91; H, 8.18; N, 3.99. Found: C, 48.04; H, 8.28; N, 3.97.

6,6′-Dideoxy-6,6′-bis[(1-hexadecylsulfonyl)amino]-α,α-trehalose (**11c**).—Compound **10c** (774 mg, 0.50 mmol) was debenzoylated by the method of **11a**, above, except that a white solid deposited after ~15 min in the alkaline MeOH solution. The collected solid was washed with Et_2O (20 mL), suspended in 1:1 MeOH–water (20 mL), and adjusted to pH 6.5 with HCl. The solid was collected (350 mg, 76%) and recrystallized from 9:1 MeOH–water (20 mL) to give a homogeneous (TLC) first crop (172 mg, 37%, mp 185–186 °C) and a lower-melting second crop (101 mg, 22%). After drying at 82 °C for 20 h, the first crop was used as the analytical sample. MS (with LiCl): *m/z* 923 $[M+Li]^+$. ^{1}H NMR (Me_2SO-d_6): δ 0.86 (t, 6 H, CH_3), 1.24, 1.34, 1.63 (4m, 24 H, 4 H, 4 H, aliphatic CH_2), 2.99 (complex m, 8 H, CH_2SO_2 + H-4 + 6-C*H*), 3.26, 3.32 (complex m, 4 H, $J_{1,2}$ 3.6, $J_{2,3}$ 9.8, $J_{2,2\text{-OH}}$ 4.8 Hz, H-2 overlapped by 6-C*H*′), 3.55 (m, 2 H, $J_{3,3\text{-OH}}$ 4.8, $J_{3,4}$ 8.5 Hz, H-3), 3.73 (m, 2 H, H-5), 4.71(d, 2 H, *J* 5.8 Hz, O*H*-2), 4.86 (d, 2 H, 3-O*H*), 4.92 (app. d, 4 H, $J_{1,2}$ 3.6, $J_{4,4\text{-OH}}$ 4.9 Hz, H-1 + 4-O*H*), 6.78 (app. t, 2 H, SO_2NHCH_2). Anal. Calcd for $C_{44}H_{88}N_2O_{13}S_2{\cdot}0.2\ H_2O$: C, 57.39; H, 9.68; N, 3.04. Found: C, 57.35; H, 10.02; N, 3.01.

6,6′-Dideoxy-6,6′-bis[(phenylsulfonyl)amino]-α,α-trehalose (**11d**).—Compound **10d** (1.02 g, 0.82 mmol) was debenzoylated by the procedure for **11a**. The crude product (500 mg, 98%) was recrystallized from a mixture of boiling EtOH (25 mL) and water (3 mL). The solid was collected, washed with EtOH, and dried at 82 °C for 20 h; yield 444 mg (86%); mp 217–218 °C. MS (with LiCl): *m/z* 627 $[M+Li]^+$. UV (EtOH): 0.1 N HCl 220 (19.0), 264 (1.77), 271 (1.46); pH 7 buffer 220 (18.5), 264 (1.56), 272 (1.28); 0.1 N NaOH 236 (sh). ^{1}H NMR (Me_2SO-d_6): δ 2.77 (m, 2 H, $J_{5,6}$ 7.3, $J_{6,6'}$ 12.8,

$J_{6,NH}$ 5.0 Hz, 6-C*H*), 2.96 (m, 2 H, $J_{3,4}$ 8.5, $J_{4,4\text{-OH}}$ 5.3, $J_{4,5}$ 10.0 Hz, H-4), 3.07 (m, 2 H, $J_{5,6'}$ 1.4 Hz, 6-C*H*′,), 3.21 (m, 2 H, $J_{1,2}$ 3.5, $J_{2,3}$ 9.7, $J_{2,2\text{-OH}}$ 6.0 Hz, H-2), 3.50 (m, 2 H, $J_{2,3}$ 9.7, $J_{3,4}$ 8.5, $J_{3,3\text{-OH}}$ 5.0 Hz, H-3), 3.70 (m, 2 H, $J_{4,5}$ 10.0 Hz, H-5), 4.64 (d, 2 H, 2-OH), 4.83 (m, 4 H, 3-OH + H-1), 4.92 (d, 2 H, 4-O*H*), 7.41 (m, 2 H, SO_2NHCH_2), 7.61, 7.81 (2m, 10 H, phenyl C*H*). Anal. Calcd for $C_{24}H_{32}N_2O_{13}S_2{\cdot}5\ H_2O$: C, 45.78; H, 5.28; N, 4.45. Found: C, 45.78; H, 5.31; N, 4.48.

*6,6′-Dideoxy-6,6′-bis[(*N,N*-didodecyl)amino]-α,α-trehalose* (**12**).—A partial solution of **7** (363 mg, 1.0 mmol) in a mixture of dodecyl iodide (652 mg, 2.2 mmol), anhyd $NaHCO_3$ (210 mg, 2.5 mmol), and dry DMAc (20 mL) was stirred under N_2 at rt for 3 days without significant reaction (TLC). The mixture was heated to 50 °C for 1 h with rapid formation of yellow color and numerous product spots. Evaporation of volatiles under high vacuum gave a crude gum (1.36 g). Column chromatography on SG with (4:1) $CHCl_3$–MeOH as eluant gave a mobile band (ca. 0.5 g) which was shown by MS to be a complex mixture of products with three to five dodecyl groups per molecule. This was followed by a very polar band of material (ca. 350 mg) whose UV behavior and salt-like characteristics suggested a mixture of quaternary iodide salts. Careful chromatography (9:1 C/M) of a 230 mg fraction from the mobile band gave the bis(didodecylamino) compound **12** as a soft waxy solid; 75 mg; mp: softens above ~75 °C. This sample was characterized only by MS and ^{1}H NMR spectra. MS: *m*/*z* 1013 $[M+H]^+$, 498 $(1/2[M-16])^+$, 366 $[CH_2N(C_{12}H_{25})_2]^+$. ^{1}H NMR ($Me_2SO$-$d_6$): δ 0.86 (t, 12 H, CH_3), 1.25 (br s, 72 H, $CH_3(CH_2)_9$), 1.47 (br s, 8 H, $CH_3(CH_2)_9CH_2$), 2.99 (m, 12 H, –CH_2NCH_2–6 plus 6-CH_2), 3.27–3.36 (br m, H-2 and H-4 overlapped by water), 3.58 (m, 2 H, H-3), 4.04 (m, 2 H, H-5), 4.88 (d, 2 H, *J* 3.5 Hz, H-1), 4.9–5.4 (very broad, OH's, best seen in integral).

Compounds **15a** and **15b** were obtained directly from **4** without isolation of intermediates **14a** and **14b**.

6,6′-Dideoxy-6,6′-bis(1-octylamino)-α,α-trehalose (**15a**).—A solution of **4** (2.00 g, 1.83 mmol) and excess *n*-octylamine (4.69 g, 36.3 mmol) in MeOH (60 mL) was refluxed with protection from moisture for 66 h. Solvent and excess amine were removed in vacuo. A solution of the residual gum in $CHCl_3$ (100 mL) was washed by shaking with water (20 mL) to which was added 3.7 mL of 1 N NaOH, followed by water. Evaporation of the dried (Na_2SO_4) organic layer gave a dark gum (1.17 g), which was applied to a SG column. Elution first with (99:1) $CHCl_3$–MeOH gave a mobile band containing *N*-octylbenzamide; elution with (15:5:1) $CHCl_3$–MeOH–concd NH_4OH gave fractions (total 303 mg) containing impure **15a**. Careful chromatography of the best fractions (230 mg) using the 15:5:1 mixture gave the analytical sample; after drying for 48 h, the yield of clear glass was 214 mg (21%); mp softens gradually above 80 °C. Attempts to induce crystallization were unsuccessful. MS: *m*/*z* 565 $[M+H]^+$, 274 $(1/2[M-16])^+$, 142 $[CH_2NHC_8H_{17}]^+$. ^{1}H NMR (Me_2SO-d_6): δ 0.86 (t, 6 H, CH_3), 1.25 (br s, 20 H, $CH_3(CH_2)_5$), 1.37 (m, 4 H, $CH_3(CH_2)_5CH_2$), 2.45 (m, 4 H, CH_2NHCH_2-6), 2.56, 2.75 (2m, 4 H, 6-CH_2), 3.04 (t, 2 H, H-4), 3.24 (dd, 2 H, H-2), 3.32 (br, OH + water), 3.53 (m, 2 H, H-3), 3.76 (complex m, 2 H, H-5), 4.4–4.8 (very br, OH's) 4.84 (d, 2 H, *J* 3.8 Hz, H-1). Anal. Calcd for $C_{28}H_{56}N_2O_9$: C, 59.55; H, 9.99; N, 4.96. Found: C, 59.23; H, 10.26; N, 4.77.

6,6′-Dideoxy-6,6′-bis(1-dodecylamino)-α,α-trehalose (**15b**).—Solid *n*-dodecylamine (14 g, excess) and **4** (1.50 g, 1.37 mmol) were combined and heated in an oil bath (65 °C) to give a clear melt, which was covered with N_2, stoppered tightly, and heated at 65 °C for 66 h. The stopper was replaced with a cold trap with a reservoir filled with dry ice. By increasing the bath temperature to 95 °C with evacuation on the oil pump, most of the excess amine was collected in the cold trap. A solution of the residue in $CHCl_3$ (100 mL) was washed by shaking with dilute base (20 mL of water to which was added 2.8 mL of 1 N NaOH), followed by water (20 mL). Evaporation of the dried organic layer gave 4.13 g of beige solid. Column chromatography on SG with (99:1) $CHCl_3$–MeOH as eluant gave a mobile band of *N*-dodecylbenzamide (2.13 g, 90%). Continued elution with (15:5:1) $CHCl_3$–MeOH–concd NH_4OH gave 0.68 g (73%) of impure **15b**. A second column on the best of these fractions (0.42 g) with the 15:5:1 mixture gave the analytical sample. After drying for 48 h, the clear glass weighed 140 mg (15%); mp softens ~85 °C. MS: *m*/*z* 677 $[M+H]^+$, 330 $(1/2[M-16])^+$, 198 $[CH_2NHC_{12}H_{25}]^+$. ^{1}H NMR (Me_2SO-d_6): 0.86 (t, 6 H, CH_3), 1.24 (br s, 36 H, $CH_3(CH_2)_9$), 1.37 (m, 4 H, $CH_3(CH_2)_9CH_2$), 2.43–2.56 (m, 4 H, CH_2NHCH_2-6), 2.57 (dd, 2 H, $J_{5,6a}$ 6.9, $J_{6a,6b}$ 12.2 Hz, H-6a), 2.77 (dd, 2 H, $J_{5,6b}$ 3.6 Hz, H-6b), 3.04 (app. t, 2 H, H-4, $J_{3,4}$ 8.8, $J_{4,5}$ 9.7 Hz, H-4), 3.24 (dd, 2 H, $J_{1,2}$ 3.6, $J_{2,3}$ 9.5 Hz, H-2), 3.32 (br, water, OH), 3.54 (app. t, 2 H, H-3), 3.77 (m, 2 H, H-5), 4.4–4.8 (very br, OH's) 4.85 (d, 2 H, H-1). Anal. Calcd for $C_{36}H_{72}N_2O_9{\cdot}5\ CH_3OH$: C, 63.26; H, 10.76; N, 4.04. Found: C, 63.10; H, 11.16; N, 4.03.

*2,3,4,2′,3′,4′-Hexa-*O*-benzoyl-6,6′-dideoxy-6,6′-bis-[[*N*-methyl-*N*-(1-octadecyl)]amino]-α,α-trehalose* (**14c**).—A solution of **4** (1.00 g, 0.92 mmol) and *N*-methyloctadecylamine (2.85 g, 10.1 mmol) in acetone (50 mL) was refluxed under an N_2 atmosphere for 66 h with minimal reaction (TLC). The acetone was evaporated and replaced with xylenes to which was added NaI (50 mg), and the solution was refluxed for 16 h under N_2. The cooled solution was evaporated and then evacuated under a cold trap (see **14b**) using a 130 °C oil bath to remove most of the excess amine. A solution of the residue (2.37 g) in $CHCl_3$ (100 mL) was washed by shaking with satd aq $NaHCO_3$ and water (100 mL

each). The dried (Na_2SO_4) organic layer was evaporated to give 2.15 g of dark gum. Column chromatography on SG with (49:1) $CHCl_3$–MeOH gave 0.84 g (61%) of **14c** with minor impurities. After confirmation of identity by mass spectral analysis (m/z 1498 $[M+H]^+$), this material was used in the deprotection step without further treatment. See **15c** below.

*2,3,4,2′,3′,4′-Hexa-*O*-benzoyl-6,6′-dideoxy-6,6′-bis-[*N,N*-bis(2-ethylhexyl)amino]-α,α-trehalose* (**14d**).—A solution of **4** (1.00 g, 0.92 mmol) and bis(2-ethylhexyl)amine (2.43 g, 10.1 mmol) in xylenes (25 mL), to which was added NaI (50 mg), was heated for 66 h 150 °C under an N_2 atmosphere. The bath was cooled to 125 °C, and the solvent and excess amine were distilled off using a trap cooled in dry ice. Column chromatography of the residue (2.06 g) on SG with $CHCl_3$ as eluant gave 0.90 g (70%) of **14d** with minor impurities. MS: m/z 1413 $[M+H]^+$. This material was used in the deprotection step without further treatment. See **15d** below.

*6,6′-Dideoxy-6,6′-bis[[*N*-methyl-*N*-(1-octadecyl)]-amino]-α,α-trehalose* (**15c**).—A suspension of **14c** (790 mg, 0.53 mmol) in MeOH (100 mL) saturated with NH_3 at 0 °C was heated at 60 °C for 18 h in a glass-lined stainless steel bomb. After cooling, the suspension was decanted into a filter funnel without disturbing some tarry matter stuck on the glass. The solid filter cake was crude **15c**; 296 mg (64%). The main component of the filtrate was benzamide as shown by TLC comparison with an authentic sample. The crude product was chromatgraphed on SG with (85:15:1) $CHCl_3$–MeOH–concd NH_4OH as eluant to give 178 mg (39%) of purified **15c**. Recrystallization from MeOH gave the analytical sample; 90 mg; mp softens gradually above ~98 °C. MS: m/z 873 $[M+H]^+$, 428 $(1/2[M-16])^+$, 296 $[CH_2N(CH_3)C_{18}H_{37}]^+$. ^{1}H NMR ($Me_2SO$-$d_6$): δ 0.86 (t, 6 H, terminal CH_3), 1.25 (br s, 60 H, $CH_3(CH_2)_{15}$), 1.38 (m, 4 H, $CH_3(CH_2)_{15}CH_2$), 2.19 (s, 6 H, $N(CH_3)$), 2.35 (m, 4 H, $CH_2N(CH_3)$), 2.39 (dd, 2 H, $J_{5,6a}$ 6.7, $J_{6a,6b}$ 13.2 Hz, H-6a), 2.65 (dd, 2 H, $J_{5,6b}$ 3.9, Hz, H-6b), 3.00 (dd, 2 H, $J_{3,4}$ 8.9, $J_{4,5}$ 9.5 Hz, H-4), 3.26 (br dd, 2 H, $J_{1,2}$ 3.7, $J_{2,3}$ 9.3 Hz, H-2), 3.59 (3d, 2 H, $J_{3,3\text{-OH}}$ 4.5 Hz, H-3), 3.88 (3d, 2 H, H-5), 4.31 (br, 2 H, 4-OH), 4.45 (d, 2 H, 3-OH), 4.82 (br, 2 H, 2-OH), 4.86 (d, 2 H, H-1). Anal. Calcd for $C_{50}H_{100}N_2O_9$: C, 68.76; H, 11.54; N, 3.21. Found: C, 68.88; H, 11.54; N, 3.28.

*6,6′-Dideoxy-6,6′-bis[*N,N*-bis(2-ethylhexyl)amino]-α,α-trehalose* (**15d**).—A solution of **14d** (892 mg, 0.63 mmol) in dry MeOH (15 mL) was treated with a solution of $NaOCH_3$ in MeOH (3 mL of 1.2 N), and the solution was stirred under N_2 at rt for 4 h. Solvent was evaporated; a solution of the residue in a large volume of $CHCl_3$ (300 mL) was shaken with water (50 mL), and the mixture was allowed to stand until the emulsion separated. The turbid organic layer was dried (Na_2SO_4), filtered, and evaporated to give 0.59 g of dark gum containing trehalose products and methyl benzoate. Column chromatography on SG with (16:4:1) $CHCl_3$–MeOH–concd NH_4OH gave 184 mg (37%) of impure **15d**. Chromatography of the best fraction (97 mg) using (9:1) $CHCl_3$–MeOH gave the analytical sample as a waxy solid, which was dried at 65 °C for 18 h; yield 77 mg (15%); mp softens gradually above ~140 °C. MS: m/z 789 $[M+H]^+$, 689 $[M-(2\text{-ethylhexyl})]^+$, 386 $(1/2[M-16])^+$. ^{1}H NMR (Me_2SO-d_6): δ 0.79 (t, 12 H, CH_3 of 2-Et), 0.86 (t, 12 H, CH_3 of hexyl), 1.10–1.47 (complex m, 36 H, alkyl CH_2), 2.11–2.34 (m, 8 H, CH_2NCH_2-6), 2.45, 2.73 (2m, 4 H, 6-CH_2), 2.91 (m, 2 H, H-4), 3.21 (m, 2 H, H-2), 3.59 (m, 2 H, H-3), 3.87 (m, 2 H, H-5), 4.39 (m, 2 H, 2-OH), 4.73 (m, 4 H, 4-OH + 3-OH), 4.91 (m, 2 H, H-1). Anal. Calcd for $C_{44}H_{88}N_2O_9$: C, 66.97; H, 11.24; N, 3.55. Found: C, 66.73; H, 11.46; N, 3.76.

*2,3,4,2′,3′,4′-Hexa-*O*-benzoyl-6,6′-dideoxy-6,6′-bis(1-hexylamino)-α,α-trehalose* (**17a**).—The ditriflate intermediate **16** was obtained by a published procedure.[34] Under an N_2 atmosphere, a solution of **16** (4.00 g, 3.25 mmol) and *n*-hexylamine (1.32 g, 13.0 mmol) in dry CH_2Cl_2 (50 mL) was stirred at rt for 48 h and then evaporated. The residual gum was triturated thoroughly with water (200 mL), and the solid was collected by filtration, washed with water, and dried. Column chromatography of the crude product (5.37 g) on SG with (49:2) $CHCl_3$–MeOH as eluant gave 2.62 g (71%) of **17a** suitable for use as an intermediate. A large homogeneous (TLC) fraction was dried at rt for 24 h for use as the analytical sample; 1.36 g; mp foam sinters above 40 °C. MS: m/z 1133 $[M+H]^+$, 558 $(1/2[M-16])^+$, 436 $[558-PhCOOH]^+$, 105 $[Bz]^+$. ^{1}H NMR ($CDCl_3$): δ 0.86 (t, 6 H, CH_3), 1.22 (complex m, 16 H, $CH_3(CH_2)_4$), 2.31 (m, 8 H, $CH_3(CH_2)_4CH_2$ + 6-CH_2), 4.01 (m, 2 H, $J_{4,5}$ 10.2, $J_{5,6}$ 4.4 Hz, H-5), 5.45 (dd, 2 H, $J_{1,2}$ 3.8, $J_{2,3}$ 10.2 Hz, H-2), 5.54 (app. t, 2 H, $J_{3,4}$ 9.6 Hz, H-4), 5.67 (d, 2 H, H-1), 6.23 (app. t, 2 H, H-3), 7.26–7.49, 7.55, 7.79, 7.91, 8.13 (m, 30 H, Bz). 6-NH absorption was very broad and was best seen in the integral in the 1.3–1.9 region. Anal. Calcd for $C_{66}H_{72}N_2O_{15}$: C, 69.95; H, 6.40; N, 2.47. Found: C, 69.98; H, 6.52; N, 2.47.

*2,3,4,2′,3′,4′-Hexa-*O*-benzoyl-6,6′-bis(1-decylamino)-6,6′-dideoxy-α,α-trehalose* (**17b**).—This product was obtained from **16** and *n*-decylamine by the same procedure as **17a**. Column chromatography (99:1 C/M) gave 1.98 g (49%) of **17b** suitable for use as an intermediate; however, a second column was required to obtain a homogeneous sample for analysis; 0.98 g (24%); mp low-melting soft glass sinters <40 °C. MS: m/z 1245 $[M+H]^+$, 614 $(1/2[M-16])^+$, 492 $[614-PhCOOH]^+$, 105 $[Bz]^+$. ^{1}H NMR ($CDCl_3$): δ 0.89 (t, 6 H, CH_3), 0.98–1.39 (m, 32 H, $CH_3(CH_2)_8$), 1.4–1.9 (very br, NH), 2.32 (complex m, 8 H, $CH_3(CH_2)_8CH_2$ + 6-CH_2),

4.02 (m, 2 H, H-5), 5.46 (dd, 2 H, H-2), 5.54 (t, 2 H, H-4), 5.68 (d, 2 H, H-1), 6.24 (t, 2 H, H-3), 7.28–7.60, 7.55, 7.79, 7.91, 8.23 (m, 30 H, Bz). Anal. Calcd for $C_{74}H_{88}N_2O_{15}$: C, 71.36; H, 7.12; N, 2.25. Found: C, 71.28; H, 7.35; N, 2.20.

2,3,4,2′,3′,4′-Hexa-O-benzoyl-6,6′-dideoxy-6,6′-bis-[(2-ethylhexyl)amino]-α,α-trehalose (**17c**).—This product was obtained from **16** (4.53 g) and (±)2-ethylhexylamine (1.05 g) by the method for **17a**. Column chromatography on SG with (99.5:0.5) $CHCl_3$–MeOH gave 2.74 g (63%) of **17c** suitable for use as an intermediate. The largest homogeneous fraction was dried at rt for 24 h; 1.67 g (38%); mp 56–58 °C with prior sintering. MS: *m/z* 1189 $[M+H]^+$, 1089 $[M-CH_3-(CH_2)_3CH(Et)]^+$, 586 $(1/2[M-16])^+$, 464 $[586-PhCOOH]^+$, 105 $[Bz]^+$. ^{1}H NMR ($CDCl_3$): δ 0.77 (m, 6 H, CH_3 of Et), 0.87 (m, 6 H, CH_3 of hexyl), 1.19 (complex m, 16 H, alkyl CH), 1.3–1.8 (very br, NH), 2.16, 2.32 (2m, 8 H, C*H*$_2$NHC*H*$_2$ superimposed on 6-CH_2), 4.02 (m, 2 H, H-5), 5.46 (dd, 2 H, H-2), 5.57 (t, 2 H, H-4), 5.67 (d, 2 H, H-1), 6.22 (t, 2 H, H-3), 7.29–7.50, 7.54, 7.77, 7.81, 8.14 (m, 30 H, Bz). Anal. Calcd for $C_{70}H_{80}N_2O_{15}$: C, 70.69; H, 6.78; N, 2.36. Found: C, 70.68; H, 6.95; N, 2.34.

6,6′-Dideoxy-6,6′-bis(1-hexylamino)-α,α-trehalose (**18a**).—A solution of **17a** (2.29 g, 2.02 mmol) in 100 mL of MeOH saturated with NH_3 at 0 °C was heated at 65 °C for 20 h in a glass-lined stainless steel bomb. The crude product (2.6 g) was chromatographed on SG with (14:6:1) $CHCl_3$–MeOH–concd NH_4OH to give **18a**; yield of clear brittle glass after drying for 6 h at 65 °C was 0.77 g (75%); mp softens 95 °C. MS: *m/z* 508 $[M+H]^+$, 246 $(1/2[M-16])^+$, 114 $[C_6H_{13}NHCH_2]^+$. ^{1}H NMR (Me_2SO-d_6): δ 0.86 (t, 6 H, CH_3), 1.25(m, 12 H, $CH_3(CH_2)_3$), 1.37 (m, 4 H, $CH_3(CH_2)_3CH_2$), 2.52 (complex m, 6 H, C*H*$_2$NH superimposed on 6-CH), 2.76 (dd, 2 H, 6-CH), 3.05 (t, 2 H, H-4), 3.24 (dd, 2 H, H-2), 3.54 (t, 2 H, H-3), 3.77 (m, 2 H, H-5), 4.4–5.1 (very br, OH's), 4.78 (br s, 2 H, 3-OH), 4.83 (d, 2 H, H-1). Anal. Calcd for $C_{24}H_{48}N_2O_9$: C, 56.67; H, 9.51; N, 5.51. Found: C, 56.62; H, 9.75; N, 5.51.

6,6′-Bis(1-decylamino)-6,6′-dideoxy-α,α-trehalose (**18b**).—Compound **17b** (0.89 g, 0.71 mmol) was combined with some previous column fractions totaling 2.2 g known to contain **17b**, but of lesser quality, and a solution of the composite sample in dry MeOH (85 mL) was treated with 1.2 N NaOMe–MeOH solution (2.60 mL) and stirred at rt for 1 h. The solution was diluted with an equal volume of water and neutralized by portionwise addition of Dowex 50W-X8 (H^+) resin. After filtration, the mixture was evaporated and re-evaporated with EtOH to give 1.92 g of pale yellow gum. MS analysis showed the desired product **18b**, 621 $[M+H]^+$, and a significant byproduct, 725 $[M+H]^+$, corresponding to **18b** with a single benzoyl group. In an attempt to remove the Bz group, if possible, a solution of the gum in liquid NH_3 (50 mL) contained in a glass-lined bomb equipped with a gas-inlet valve was allowed to stand at rt for 24 h, and then the NH_3 was allowed to evaporate overnight. Removal of the benzoyl group was not complete, but the byproduct spot was considerably diminished (TLC). Column chromatography on SG with (15:5:1) $CHCl_3$–MeOH–concd NH_4OH gave 0.55 g of mixed **18b** and monobenzoyl byproduct, followed by 0.62 g of **18b** with a barely detectable trace of sodium (or ammonium) benzoate. The latter was removed by shaking a $CHCl_3$ solution with satd $NaHCO_3$ solution and with water. The glassy crust recovered from the $CHCl_3$ layer was crystallized by extraction into ~100 mL of boiling acetone in a Soxhlet apparatus. The waxy solid was dried at 82 °C for 24 h; 296 mg; mp sinters 115 °C. MS: *m/z* 621 $[M+H]^+$, 302 $(1/2[M-16])^+$, 170 $[C_{10}H_{21}NHCH_2]^+$. ^{1}H NMR (Me_2SO-d_6): δ 0.86 (t, 6 H, CH_3), 1.25 (br s, 28 H, $CH_3(CH_2)_7$), 1.37 (m, 4 H, $CH_3(CH_2)_7CH_2$), 2.52, 2.75 (2m, 8 H, C*H*$_2$NHC*H*$_2$ and 6-CH_2), 3.05 (t, 2 H, H-4), 3.24 (dd, 2 H, H-2), 3.55 (m, 2 H, H-3), 3.76 (m, 2 H, H-5), 3.9-4.8 (very br, OH's), 4.84 (m, 2 H, H-1). Anal. Calcd for $C_{32}H_{64}N_2O_9$: C, 61.91; H, 10.39; N, 4.51. Found: C, 61.84; H, 10.45; N, 4.43.

6,6′-Dideoxy-6,6′-bis[(2-ethylhexyl)amino]-α,α-trehalose (**18c**).—A solution of NaOMe–MeOH (2.6 mL of 1.2 N) was added to a solution of **17c** (1.61 g, 1.35 mmol) in dry MeOH (20 mL). The reaction mixture was stirred under N_2 at rt for 1 h and then treated with cation exchange resin with subsequent workup as described for **18b**. Column chromatography of the crude product (0.86 g) on SG with (80:20:1) $CHCl_3$–MeOH–concd NH_4OH gave a rough separation of **18c** (282 mg, 37%) from a byproduct shown to be a (mono) *N*-benzoyl derivative of **18c** (see Section 2). A second column gave the analytical sample as a brittle foam, which was dried at 65 °C for 30 h; 110 mg (14%); mp softens ~158 °C. MS: *m/z* 565 $[M+H]^+$, 274 $(1/2[M-16])^+$, 142 [alkylNHCH$_2$–]$^+$. ^{1}H NMR (Me_2SO-d_6): δ 0.85 (m, 12 H, CH_3 of Et and hexyl), 1.10–1.42 (m, 18 H, alkyl CH), 2.38, 2.47 (2m, 4 H, CH(Et)C*H*$_2$NH), 2.59, 2.76 (2dd, 4 H, 6-CH_2), 3.06 (t, 2 H, H-4), 3.24 (dd, 2 H, H-2), 3.55 (m, 2 H, H-3), 3.78 (m, 2 H, H-5), 4.2–5.2 (very br, OH's), 4.79 (d, 2 H, 3-OH), 4.86 (d, 2 H, H-1). Anal. Calcd for $C_{28}H_{56}N_2O_9$: C, 59.55; H, 9.99; N, 4.96. Found: C, 59.42; H, 10.17; N, 4.87.

2,3,4,2′,3′,4′,6′-Hepta-O-benzoyl-6-O-(trifluoromethylsulfonyl)-α,α-trehalose (**19**).—Hepta-*O*-benzoyltrehalose had been obtained by a literature procedure,[34] and a 4.10 g sample was converted to **19** in 99% crude yield[34] for their compound **39**. MS (with LiCl): *m/z* 1209 $[M+Li]^+$, 607, 579 [unsymmetrical glucopyranosyl moieties from fragmentation of the glycosidic

bond]$^+$, 105 [Bz]$^+$. ^{1}H NMR ($CDCl_3$): 3.86, 4.00 (2m, 4 H, 6-CH_2), 4.28 (m, 2 H, H-5), 5.49 (m, 3 H, H-2's and H-4 of ring with 6-OTf), 5.69 (m, 1 H, H-4 of ring with 6-OBz), 5.70, 5.77 (2d, 2 H, H-1's), 7.24–8.15 (m's, 35 H, Bz). Anal. Calcd for $C_{62}H_{49}F_3O_{20}S$: C, 61.90; H, 4.10. Found: C, 61.94; H, 3.74.

2,3,4,2′,3′,4′6′-Hepta-O-*benzoyl-6-deoxy-6-[(2-ethylhexyl)amino]-α,α-trehalose* (**20**).—A solution of **19** (4.46 g, 3.71 mmol) and (±)2-ethylhexylamine (1.01 g, 7.8 mmol) in CH_2Cl_2 (50 mL) was allowed to stand at rt for 20 h and then evaporated. The crude product was chromatographed on SG with $CHCl_3$ as eluant. The leading and trailing edges of the main product band (total 4.2 g, 96%) were rejected because of trace contaminants. The homogeneous middle cut gave the analytical sample as a brittle foam after drying at rt for 18 h; 2.29 g (52%); mp sinters ~60 °C. MS: *m/z* 1182 $[M+H]^+$, 586, 579 [unsymmetrical glucopyranose fragments]$^+$, 464 $[586-BzOH]^+$, 105 $[Bz]^+$. ^{1}H NMR ($CDCl_3$): δ 0.75(m, 3 H, CH_3 of Et), 0.86 (m, 3 H, CH_3 of hexyl), 1.0–1.3 (br m, 9 H, alkyl CH), 1.4–1.8 (very br, NH), 2.14, 2.27 (2m, 4 H, CH(Et)CH_2NH plus 6-CH_2 of ring with alkylamine), 3.92, 4.03 (2m, 3 H, 6-BzOCH_2 plus H-5), 4.25 (m, 1 H, H-5 of ring with 6-BzOCH_2), 5.46 (dd, 2 H, H-2's), 5.57, 5.64 (2d, 2 H, H-4's), 5.72 (m, 2 H, H-1's), 6.26 (m, 2 H, H-3's), 7.15–8.18 (m's, 35 H, Bz). UV (EtOH): 0.1 N HCl 233 (80.2), 275 (11.3), 284 (sh); pH 7 buffer 242 (80.3), 276 (sh), 283 (sh); 0.1 N NaOH 238 (88.4), 276 (sh), 283 (sh). Anal. Calcd for $C_{69}H_{67}NO_{17}$: C, 70.10; H, 5.71; N, 1.18. Found: C, 70.11; H, 5.46; N, 1.16.

6-Deoxy-6-(2-ethylhexyl)amino-α,α-trehalose (**21**).—A solution of NaOMe in MeOH (2.3 mL of 1.2 N) was added to a suspension of **20** (4.02 g, 3.4 mmol) in dry MeOH (225 mL), and the mixture was stirred under N_2 for 2 h and then evaporated. The residue was washed by trituration and decantation with hexane (2 × 50 mL), and the dried crude product (1.75 g) was chromatographed on SG. Elution with (12:8:1) $CHCl_3$–MeOH–concd NH_4OH gave 190 mg (10%) of the *N*-benzoyl byproduct followed by **21** (1.32 g, 86%). Recrystallization of the latter from boiling acetone gave a granular white solid, which was dried at rt for 18 h; yield 1.13 g (73%); mp sinters ~106 °C. MS: *m/z* 454 $[M+H]^+$. ^{1}H NMR (Me_2SO-d_6): δ 0.84 (m, 6 H, CH_3's of Et and hexyl), 1.1–1.4 (br m, 9 H, alkyl CH), 2.38, 2.48 (2m, 2 H, CH(Et)CH_2NH), 2.60 (dd, 1 H, $J_{5,6a}$ 6.9, $J_{6a,6b}$ 12.6 Hz, 6-CH_a), 2.77 (dd, 1 H, $J_{5,6b}$ 3.5 Hz, 6-CH_b), 3.05 (dd, 1 H, $J_{3,4}$ 8.6, $J_{4,5}$ 10.0 Hz, H-4), 3.14 (3d, 1 H, $J_{3',4'}$ 8.9, $J_{4',5'}$ 9.9, $J_{4',4'\text{-OH}}$ 5.1 Hz, H-4′), 3.24 and 3.25 (2m, 2 H, $J_{1,2}$ $J_{1',2'}$ 3.6, $J_{2,3 \text{ or } 2',3'}$ 9.7, $J_{2',3' \text{ or } 2,3}$ 9.6 Hz, H-2 and H-2′), 3.40–3.61 (complex m, 4 H, 6′-CH_2OH + H-3 + H-3′), 3.65 (m, 1 H, H-5′), 3.79 (m, 1 H, H-5), 4.36 (t, 1 H, *J* 5.8 Hz, 6′-CH_2O*H*)*, 4.62 (d, 1 H, $J_{2',2'\text{-OH}}$ 6.3 Hz, 2′-OH)*, 4.77 (m, 3 H, 2-OH + 4-OH + 4′-OH, *J* 4.7, 5.1, 4.7 Hz),† 4.87 and 4.88 (2m, 2 H, H-1 and H-1′). Anal. Calcd for $C_{20}H_{39}NO_{10}$: C, 52.97; H, 8.67, N, 3.09. Found: C, 52.71; H, 8.55; N, 3.02.

Intermediates **23a**–**d** were all obtained by a literature method,[36] which is illustrated below for **23a**.

Hexaneimidic acid, methyl ester, hydrochloride ***23a***.—Under an N_2 atmosphere, a solution of hexanenitrile (2.71 g, 27.8 mmol) and dry MeOH (5 mL) in dry Et_2O (40 mL) was cooled in an ice–water bath, and gaseous HCl was bubbled in for 15 min. The mixture was stored in the freezer overnight. Solvents were evaporated, and the residue was triturated thoroughly with *cold* Et_2O (20 mL). The slurry of white crystals was filtered under N_2 pressure, washed with cold Et_2O, and dried in vacuo over P_2O_5; yield 4.18 g (91%). Used 'as is' without characterization.

Intermediates **23b**,**c**, and **d** were obtained from the appropriate nitriles by the same method as **23a**. An ^{1}H NMR spectra was obtained for methyl dodecaneimidate hydrochloride **23b**. ^{1}H NMR (Me_2SO-d_6): δ 0.86 (t, 3 H, dodecyl CH_3), 1.25 (br s, 16 H, $CH_3(CH_2)_8$), 1.59 (m, 2 H, $(CH_2)_8CH_2$), 2.62 (t, 2 H, $CH_2C{=}NH_2^+$), 4.07 (s, 3 H, OCH_3), 7.18, 7.35, 7.52 (3s, 3 H, *J* 49.4 Hz, NH).

6,6′ - Dideoxy - 6,6′ - bis[(1 - iminohexyl)amino] - α,α - trehalose (**24**), *mixture with 6,6′-dideoxy-6-(1-iminohexyl)amino-6′-(1-oxohexyl)amino-α,α-trehalose* (**25**), *compound with acetic acid [10:1:25].*—With protection from moisture, a partial solution of **7** (732 mg, 2.0 mmol) in dry MeOH (250 mL) was heated to boiling and then cooled to ~50 °C to increase solubility. Freshly activated Linde 3 Å molecular sieve (6 g) was added, followed by methyl hexaneimidate hydrochloride (1.32 g, 8.0 mmol) and dry Amberlite IRA-400 anion exchange resin (2.0 g, OH^- form, nominal 4 mmol/g exchange capacity). The mixture was stirred at rt under N_2 for 3 days. TLC showed two product spots, but much unreacted starting materials, so the mixture was heated in a 50 °C oil bath for 3 days more. The suspended solids were removed by filtration, and the filter cake (resin and sieves) was washed liberally with MeOH. Evaporation of the filtrate gave a residue that was washed by trituration and decantation with hexane (2 × 25mL) to remove some imidate ester free base. Flash column chromatography of the dried residue (1.2 g) on SG with (20:20:1) $CHCl_3$–MeOH–HOAc gave a mobile band of mixed organic and inorganic material whose main organic component (28 mg) was **25** by MS: *m/z* 536 $[M+H]^+$; TLC (20:20:1): R_f 0.58. Continued

† These clearly-defined OH peaks only account for five of the seven OH protons; however, the region 4.30–4.82 integrates for seven protons, and very broad absorption (tentatively assumed to be the 3- and 3′-OH's) is also evident in the baseline. All absorption in this region disappears upon exchange with D_2O.

elution gave a similar band whose main organic component was **24** by MS: m/z 535 $[M+H]^+$; TLC (20:20:1): R_f 0.17. A turbid solution of **24** in water (5 mL) was filtered, and the filtrate was applied to a Bio-Beads™ SM-4 resin column (20–50 mesh, 40 mL packed volume). Elution with water removed most of the inorganic matter. Elution with (9:1) water–MeOH and finally with (1:1) gave 190 mg of **24** and leached resin. This solid was stirred vigorously into a fine suspension in Et_2O, filtered under N_2 pressure, and the solid was washed twice with Et_2O and dried for 24 h; yield 111 mg (8% as the composition given below); mp ~125 °C with prior sintering. MS: m/z 535 $[M+H]^+$, 259 (1/2$[M-16]$)$^+$, weak 536 $[M+H]^+$ of **25**. ^{1}H NMR of **24** (Me_2SO-d_6): δ 0.87 (t, 6 H, CH_3), 1.27 (m, 8 H, $CH_3(CH_2)_2$), 1.57 (m, 4 H, $CH_3(CH_2)_3CH_2$), 2.39 (m, 4 H, CH_2C=NH), 3.05 (t, 2 H, H-4), 3.26 (m, 4 H, H-2, superimposed on 6-CH), 3.42 (br s, 2 H, 6-CH), 3.56 (t, 2 H, H-3), 3.93 (m, 2 H, H-5), 4.90 (d, 2 H, H-1), OH and NH very broad, best seen in integral and baseline. Very strong singlet at 1.70 (CH_3 of HOAc). A number of small peaks, some barely visible above the baseline, correspond to those of component **25** of the 28 mg byproduct band. Anal. Calcd for $C_{24}H_{46}N_4O_9$·0.1 $C_{24}H_{45}N_3O_{10}$·2.5 $C_2H_4O_2$: C, 51.08; H, 8.26; N, 8.16. Found: C, 51.03; H, 8.42; N, 7.88.

6-Amino-6,6′-dideoxy-6′-[(1-iminododecyl)amino]-α,α-trehalose (**26**) *and 6,6′-dideoxy-6,6′-bis[(1-iminododecyl)amino]-α,α-trehalose* (**27**).—Linde 3 Å molecular sieves (6 g) were added to a partial solution of **7** (145 mg, 0.36 mmol) and methyl dodecaneimidate hydrochloride **23b** (161 mg, 0.7 mmol) in MeOH (140 mL), and the mixture was stirred at rt for 48 h. The pulverized sieves were filtered off and washed with MeOH; the filtrate was evaporated to give a solid mixture of at least six components (TLC). Column chromatography on 70–230 mesh SG with (10:2:3) *n*-BuOH–HOAc–water as eluant gave, after evaporation of solvent and lyophilization of a water solution of the residues, 14 mg (~6%) of **26** identified only by MS: m/z 522 $[M+H]^+$ and 24 mg (~9%) of **27**. MS: m/z 703 $[M+H]^+$. ^{1}H NMR (D_2O): δ 0.78 (t, 6 H, CH_3), 1.19 (br s, 32 H, $CH_3(CH_2)_8$), 1.58 (br m, 4 H, $CH_3(CH_2)_8CH_2$), 1.81(s, H*OAc*), 2.45 (br m, 4 H, $CH_2C(=NH_2^+)$), 3.23 (app. t, 2 H, $J_{3,4}+J_{4,5}$ 18.8 Hz, H-4), 3.40–3.48 (2m, 4 H, H-5 + H-2), 3.59 (m, 2 H, H-5′), 3.76 (m, 2 H, $J_{2,3}+J_{3,4}$ 18.5 Hz, H-3), 3.89 (m, 2 H, H-4), 5.00 (br s, 2 H, $J_{1,2}$ 2.8 Hz, H-1).

6,6′-Bis[[[benzyloxycarbonylamino-N-benzyloxycarbonyl]iminomethyl]amino]-6,6′-dideoxy-α,α-trehalose (**31**).—*N,N′*-Bis(benzyloxycarbonyl)-*S*-methylisothiourea (**30**) was obtained as a clear colorless oil in 99% crude yield from commercial *S*-methylisothiourea hemisulfate (10.0 g, Sigma) and benzyl chloroformate by the method of Tian et al.[36] MS: m/z 359 $[M+H]^+$. A solution of diaminotrehalose **7** (716 mg, 2.0 mmol) and excess **30** (1.86 g, 5.2 mmol) in dry DMF (120 mL) was stirred under N_2 at rt for 6 days, although the stench of methyl mercaptan became obvious in a few hours. Volatiles were evaporated, and the solid residue was stirred with Et_2O (100 mL) and collected by filtration. Column chromatography of the crude product (1.43 g, 75%) with (9:1) $CHCl_3$–MeOH as eluant gave a white solid suitable for use as an intermediate; 1.00 g (52%); mp 128–130 °C. MS: m/z 961 $[M+H]^+$ corresponding to the desired product with four CBz groups and a weak 827 $[M+H]^+$ corresponding to a similar product with three CBz groups. Since all CBz groups would be removed in the final step, this latter component was of no consequence. ^{1}H NMR (Me_2SO-d_6): δ 3.03 (m, 2 H, H-4), 3.26 (complex m, 4 H, H-2 superimposed on 6-CH), 3.53 (m, 2 H, H-3), 3.80 (m, 2 H, 6-CH′), 3.92 (m, 2 H, H-5), 4.91 (2d, 4 H, 2-OH + 3-OH), 4.96 (d, 2 H, J 3.9 Hz, H-1), 5.03 (s, 4 H, CH_2 of CBz), 5.20 (m, 6 H, CH_2 of CBz superimposed on 4-OH), 7.37 (complex m, 20 H, aromatic CH of CBz), 8.54 (m, 2 H, 6-CH_2N*H*), 11.53 (m, 2 H, CBzN*H*). Water noted. Anal. Calcd for $C_{46}H_{52}N_6O_{17}$·0.1 $C_{38}H_{46}N_6O_{15}$·2 H_2O: C, 57.12; H, 5.49; N, 8.83. Found: C, 57.14; H, 5.37, N, 8.81.

6,6′-Bis[(aminoiminomethyl)amino]-6,6′-dideoxy-α,α-trehalose (6,6′-dideoxy-6,6′-diguanidino-α,α-trehalose) (**32**).—A suspension of **31** (880 mg, 0.84 mmol) in MeOH (100 mL) was warmed until solution was obtained and then allowed to cool without stirring. $CHCl_3$[38] (1 mL), water (1 mL), and 10% Pd–C (200 mg) were added, and the mixture was hydrogenated at atmospheric pressure until H_2 uptake ceased after ~1.5 h. The mixture was filtered through a thin Celite pad under N_2 pressure, and the clear colorless filtrate was evaporated. A solution of the residue in MeOH (25 mL) was treated slowly dropwise with Et_2O (250 mL), and the deposit of dense white crystalline dihydrochloride salt was collected by filtration under N_2 pressure, washed with Et_2O, and dried at 100 °C for 24 h; yield 412 mg (98%); mp sinters above ~120 °C then decomposes with foaming at 180 °C. MS: m/z 425 $[M+H]^+$, 204 (1/2$[M-16]$)$^+$. ^{1}H NMR (Me_2SO-d_6): δ 3.06 (t, 2 H, H-4), 3.33 (complex m, 6 H, 6-CH_2 + H-2), 3.57 (t, 2 H, H-3), 3.92 (m, 2 H, H-5), 5.00 (d, 2 H, J 3.9 Hz, H-1), 5.7–7.5 (very br, H^+, NH, OH). Anal. Calcd for $C_{14}H_{28}N_6O_9$·2 HCl·0.2 H_2O: C, 33.57; H, 6.12; Cl, 14.15; N, 16.78. Found: C, 33.61; H, 6.00; Cl, 14.02; N, 16.69.

Growth inhibitory assays.—The MIC was determined using either a colorimetric microdulition broth assay or a macrodilution broth assay. The microdilution broth assay is described elsewhere;[37,38]; the macrodilution broth assay was similar except that the assay volume was 1 mL and 12 × 75 mm glass tubes were used instead of microtiter plates. *M. tuberculosis* H37Ra was obtained from the American Type Culture Collection,

Manassas, VA (ATCC 25177); the *M. avium* strains were clinical isolates obtained from the National Jewish Center for Immunology and Respiratory Diseases, Denver, CO.

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