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Synthesis of mono- and dideoxygenated α,α-trehalose analogs

Fiona L. Lin,[a] Herman van Halbeek[a] and Carolyn R. Bertozzi[a,b,c,d,*]

[a]*Department of Chemistry, University of California, Berkeley, CA 94720, United States*
[b]*Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, United States*
[c]*Howard Hughes Medical Institute, University of California, Berkeley, CA 94720, United States*
[d]*The Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, United States*

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Abstract—In this work, we describe the synthesis and NMR characterization of four mono- and four dideoxygenated analogs of α,α-D-trehalose. The symmetrical (2,2′-, 3,3′-, 4,4′- and 6,6′-) dideoxy analogs were obtained via selective protection and subsequent radical deoxygenation of the desired hydroxyl group set. The unsymmetrical (2′-, 3′-, 4′- and 6′-) monodeoxy analogs were synthesized by desymmetrization of α,α-trehalose and subsequent deoxygenation under radical conditions. Complete assignment of all ^{1}H and ^{13}C resonances in the spectra of these deoxytrehaloses was achieved through the extensive use of 2D $\{^{1}H,^{1}H\}$ and $\{^{1}H,^{13}C\}$ correlation NMR experiments. The synthesis of these trehalose analogs sets the stage for future biochemical and NMR-based studies to probe the substrate interactions of trehalose with the recently identified mycobacterial sulfotransferase Stf0.

Keywords: Deoxytrehalose; Deoxygenation; Mycobacterial sulfotransferase; NMR spectroscopy

1. Introduction

Trehalose [α-D-Glc-(1↔1)-α-D-Glc] (**1**, Fig. 1) is a symmetrical, non-reducing disaccharide found in many organisms from plants and insects to microbes.[1] In many biological systems, trehalose is associated with fortification of cells against various types of physiological stresses including osmotic shock,[1] heat and cold shock,[2] as well as oxygen radicals.[3] While produced non-constitutively in many organisms in response to changing environment, interestingly, trehalose biosynthesis in mycobacteria is constitutive and subjected to constant turnover,[4,5] suggesting a utility for trehalose in these bacteria beyond stress response. Mycobacterial trehalose is found in both the cytoplasm and the cell wall,[6] where it is incorporated into a variety of glycolipids including trehalose 6,6′-dimycolate (cord factor), sulfolipids (acylated trehalose-2-sulfate) and lipooligosaccharides.[7] Among the known trehalose-containing metabolites, sulfolipid-1 (SL-1) (**2**, Fig. 1) is the most abundant sulfur-containing lipid in *Mycobacterium tuberculosis* (accounting for up to 0.7% of cellular dry weight).[8–13] The high abundance of SL-1 suggests that it plays crucial roles in the survival and/or virulence of *M. tuberculosis*. Furthermore, in vitro cell-based assays have demonstrated a strong correlation between the presence of SL-1 and a range of host immunological responses.[14–17]

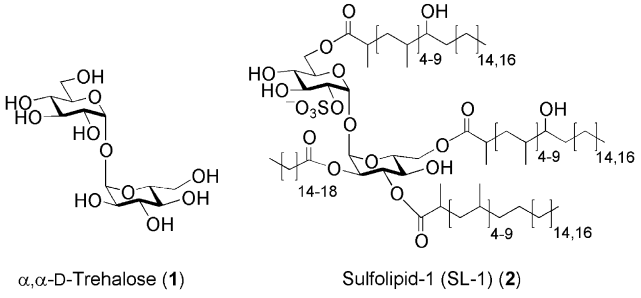

Figure 1. Structures of α,α-trehalose (**1**) and sulfolipid-1 (**2**).

First discovered by Dubos and Middlebrook,[8] and later characterized by Goren and co-workers,[10–13] SL-1 consists of a trehalose core that is acylated with three distinct lipids at the 2-, 3-, 6- and 6′-positions and is

* Corresponding author. Tel.: +1 510 6431682; fax: +1 510 6432628; e-mail: crb@berkeley.edu

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sulfated at the 2′-position. Although SL-1 was discovered and characterized several decades ago, the molecular details of SL-1 biosynthesis have not yet been fully elucidated. Our laboratory has recently identified a novel mycobacterial sulfotransferase, Stf0, which initiates SL-1 biosynthesis by the transfer of a sulfuryl group from 3′-adenosyl-5′-phosphosulfate (PAPS) to the 2′-hydroxyl group of trehalose (Scheme 1).[18] The lack of SL-1 precursors in Stf0 knockout mutants provided evidence that this is the obligate first step in SL-1 biosynthesis. Given that trehalose is not biosynthesized in humans, disruption of SL-1 biosynthesis presents a potential target for anti-tubercular drug development. Towards this goal, a detailed understanding of Stf0 substrate recognition is critical. Herein we present the synthesis of eight deoxygenated trehalose analogs (Fig. 2), which set the stage for future biochemical studies of Stf0 substrate recognition.

The chemical manipulation of trehalose, as a C_2-symmetrical, non-reducing disaccharide, presents two major challenges, namely: (1) construction of the α,α-1,1 glycosidic linkage, and (2) differentiation between the chemically similar secondary hydroxyl groups. Synthetic trehalose analogs that have been reported to date primarily contain modifications of the C4 and C6 hydroxyl groups. Synthetic manipulation of these positions is facilitated by the use of the benzylidene protecting group, and the unique reactivity of the primary C6 hydroxyl residue can furthermore be selectively targeted.[19–28] Reports on the modifications of the C2 and C3 hydroxyl groups are rare, except for a series of papers published by Hough, Richardson and co-workers on the use of trehalose diepoxides in the synthesis of the 2,2′- and 3,3′-dideoxytrehalose analogs.[29–31] These compounds, however, do not possess the native stereochemical configuration of trehalose, but rather have an inverted stereochemistry at the carbon adjacent to the deoxygenated site. The work reported herein represents the first systematic approach to synthesize a complete panel of mono- and symmetrical dideoxygenated trehalose analogs (Fig. 2).

2. Results and discussion

We considered two synthetic strategies for preparation of the target deoxytrehalose analogs. Dimerization of

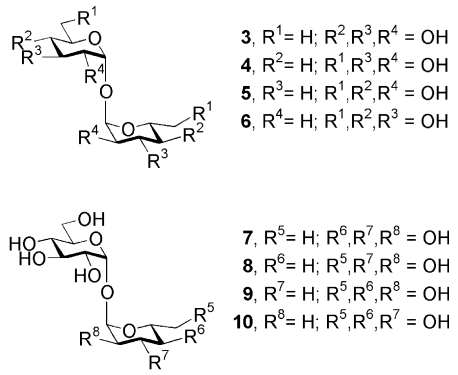
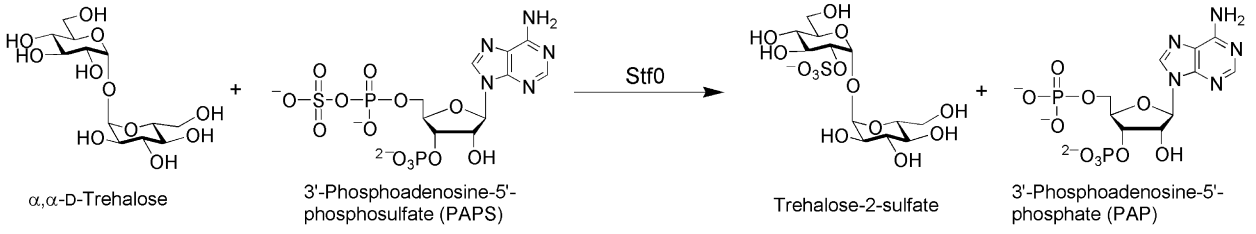


Figure 2. Synthetic targets: symmetrical dideoxygenated trehalose analogs **3**–**6** and unsymmetrical monodeoxygenated trehalose analogs **7**–**10**.

fully protected and appropriately deoxygenated glucosyl donors and acceptors would allow for a convergent approach, but requires the construction of the synthetically challenging α,α-1,1 linkage and also entails lengthy preparation of the individual deoxy-glucosyl building blocks.[32] An alternative approach involves desymmetrization of commercially available α,α-trehalose, requiring judicious choice of orthogonal protecting groups in order to isolate the desired set of hydroxyl groups. Removal of the hydroxyl functionality can then be conveniently achieved by using Robins' radical deoxygenation conditions.[33,34] Herein we employ the latter approach to efficiently construct the proposed panel of deoxygenated trehalose analogs.

2.1. Synthesis of 6,6′-dideoxy (3) and 6′-monodeoxy (7) analogs

The syntheses of 6,6′-dideoxytrehalose (**3**) and 6-deoxytrehalose (**7**) have been previously reported. The reported synthesis of **7** relies on the selective mono-bromination of α,α-trehalose;[28] unfortunately, in our hands, we only obtained the dibrominated product when using this procedure. While the 6,6′-dideoxy analog **3** could be prepared from the dibrominated compound, or via the selective sulfonylation of the 6- and 6′-hydroxyl groups of α,α-trehalose dihydrate, followed by conversion to the iodide and subsequent reductive dehalogenation,[26] we chose an alternative synthetic route that



Scheme 1. Stf0-catalyzed sulfation of trehalose in SL-1 biosynthesis.

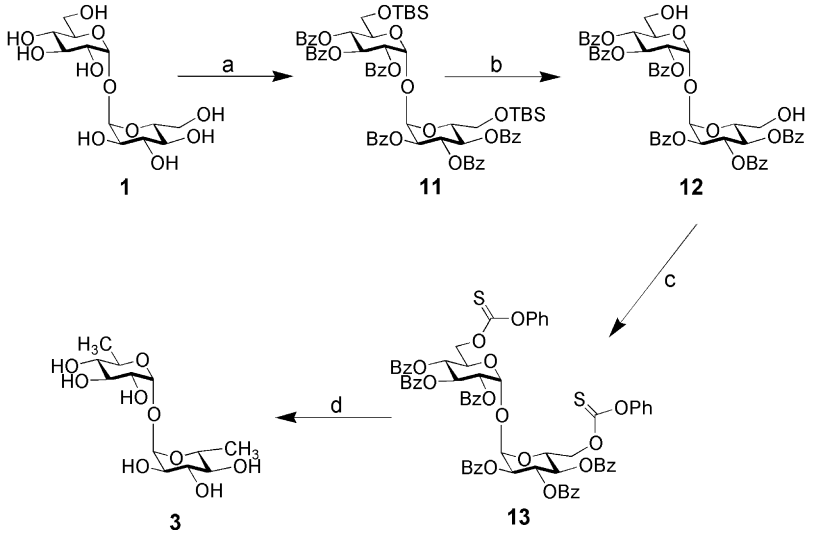
enables preparation of both the mono- and also the dideoxygenated products from a common intermediate.

The synthesis of compound **3** began with selective silylation of the primary alcohols of trehalose (Scheme 2). Global protection of the remaining hydroxyl groups as benzoyl esters afforded **11**, and removal of the two silyl ethers using TBAF provided 6,6′-diol **12**. Compound **12** was then activated as thionocarbonate **13** and subjected to radical deoxygenation conditions. The reaction yields of the radical cleavage of the primary thionocarbonate were highly variable, with multiple side products observed as shown by TLC analysis. Removal of all benzoyl protecting groups provided 6,6′-dideoxytrehalose (**3**) (Scheme 2).

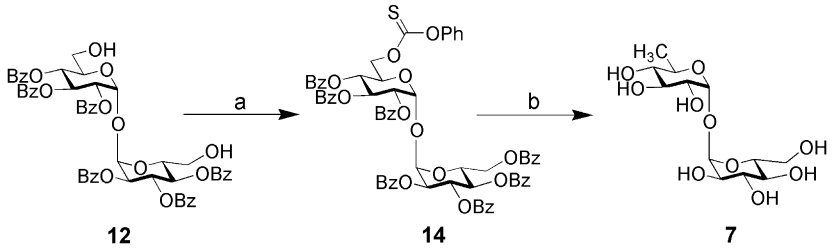
The synthesis of 6′-deoxytrehalose (**7**) also utilized diol **12**. Using 1 equiv of benzoyl chloride, diol **12** was mono-acylated (Scheme 3). The low overall yield from **12** to **14** was predicated from overbenzoylation of **12**. Nevertheless, the remaining free 6′-hydroxyl group was removed as described for **13** via thionocarbonate **14** to provide 6-deoxytrehalose (**7**) in moderate yield.

2.2. Synthesis of 4,4′-dideoxy (4) and 4′-monodeoxy (8) analogs

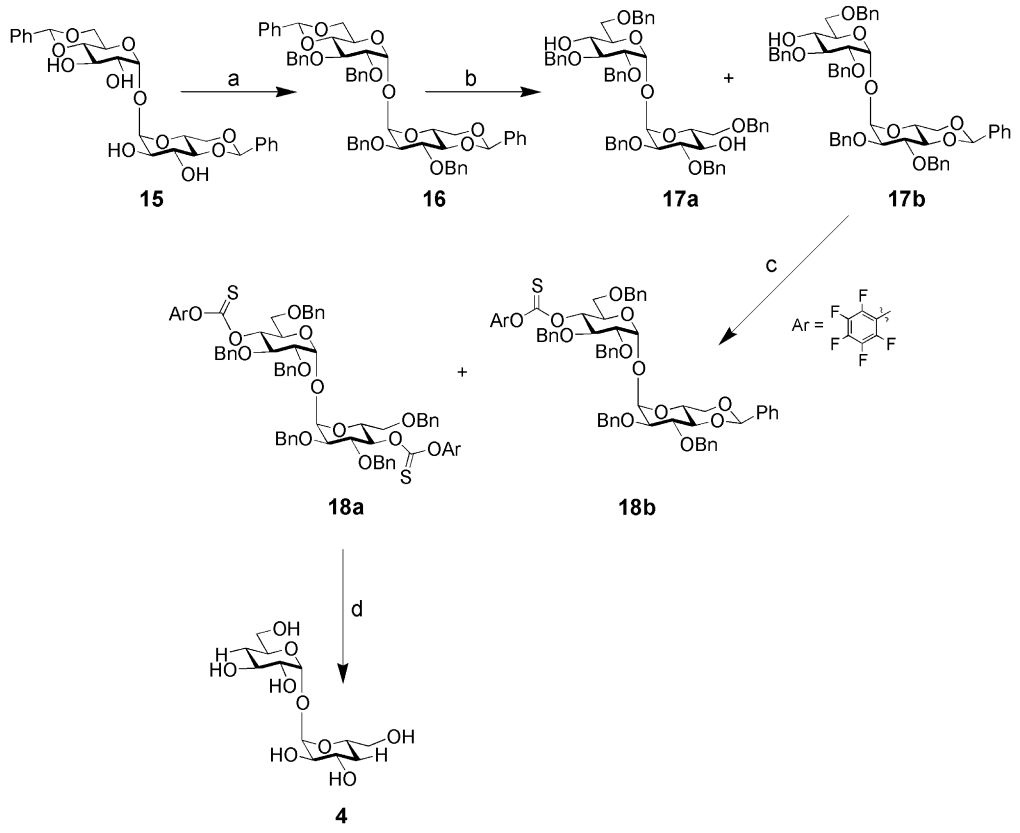
The known dibenzylidene acetal of trehalose[35] **15** served as a common intermediate in the syntheses of the 4,4′-; 4-; 2,2′- and 3,3′-deoxytrehalose analogs. For the symmetrical 4,4′-dideoxy analog **4**, we employed regioselective reductive cleavage[36] of benzylidene acetal **16** using $TfOH/NaCNBH_3$ to expose the 4- and 4′-hydroxyl groups (Scheme 4). The reaction was very sluggish, and complete conversion to **17a** could not be achieved even with the addition of large excesses (>15 equiv) of reagents. The use of HCl instead of TfOH offered no improvement. By TLC analysis, both the mono- and dibenzylidene reduction products could be observed, albeit with very similar R_f values. Since the monoreduction product **17b** could be used to synthesize the 4′-monodeoxy analog **8**, we isolated both compounds. Although we were unable to fully separate **17a** and **17b** even after extensive column chromatography, subjecting the mixture to thionocarbonylation afforded **18a** and **18b** that are separable by silica gel chromato-



Scheme 2. Reagents: (a) (i) TBSCl, Py (ii) BzCl, Py (92% over two steps); (b) TBAF, 1:9 AcOH–THF (72%). (c) PTC-Cl, DMAP, MeCN; (d) (i) Bu_3SnH, AIBN (cat.), PhMe; (ii) NaOMe, MeOH (32% over three steps).



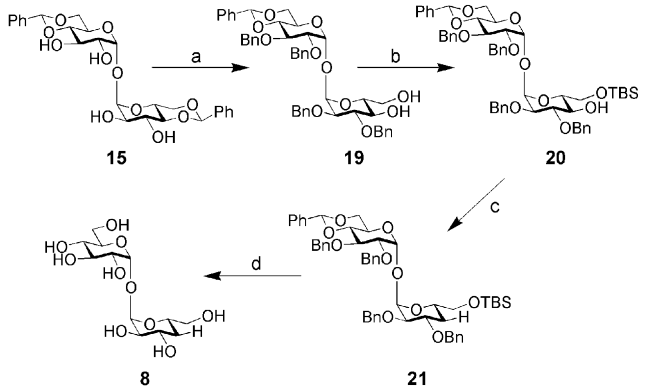
Scheme 3. Reagents: (a) (i) BzCl (1.0 equiv), Py; (ii) PTC-Cl, DMAP, MeCN (10% over two steps); (b) (i) Bu_3SnH, AIBN (cat.), PhMe; (ii) NaOMe, MeOH (60% over two steps).



Scheme 4. Reagents: (a) NaH, BnBr, DMF (90%); (b) $NaCNBH_3$, TfOH, THF; (c) pentafluorophenyl thionochloroformate, DMAP, MeCN (10% for **18a**, 10% for **18b** over two steps); (d) (i) Bu_3SnH, AIBN (cat.), PhMe; (ii) H_2, $Pd(OH)_2/C$, MeOH (36% over two steps).

graphy (Scheme 4). Deoxygenation of **18a** and deprotection provided 4,4′-dideoxytrehalose **4** in 36% yield over two steps.

Although monobenzylidene acetal **17b** could be obtained from the incomplete cleavage of dibenzylidene **16** (Scheme 4), it was difficult to achieve consistent yields for the conversion of **16** to **17b**. Therefore, an alternative route was devised for the targeted synthesis of 4-monodeoxytrehalose **8** (Scheme 5). Subjecting dibenzylidene



Scheme 5. Reagents: (a) (i) BnBr, NaH, DMF; (ii) 2:8 TFA–MeOH, 2 h (40% over two steps); (b) TBSCl, Et_3N, CH_2Cl_2 (80%); (c) (i) PTC-Cl, DMAP, MeCN; (ii) Bu_3SnH, AIBN (cat.), PhMe (58% over two steps); (d) H_2, $Pd(OH)_2/C$, 1:9 AcOH–MeOH (52%).

16 (Scheme 4) to a brief acidic, rather than reductive, cleavage removed only one of the two benzylidene acetals,[19] furnishing **19** as the major product. Silylation of the primary alcohol of **19** provided **20** in 80% yield. Interestingly, thionocarbonylation of the 4′-hydroxyl of **20** did not proceed to completion even after multiple additions of phenyl thionochloroformate (PTC-Cl) and DMAP, presumably due to the bulky silyl group at the nearby 6′-position. Nonetheless, cleavage of the semi-purified thionocarbonate under radical deoxygenation conditions afforded the 4′-deoxygenated product **21** in 58% yield over two steps. Simultaneous removal of benzyl and silyl ethers by acidic hydrogenolysis provided the final monodeoxy analog **8**.

2.3. Synthesis of 3,3′-dideoxy (5) and 2,2′-dideoxy (6) analogs

For the synthesis of the 3,3′-dideoxy analog **5**, we first attempted dimerization of 2-acetoxyglucal via the in situ generation of glucosyl iodide as previously reported.[37] However, the starting material and product have similar R_f values, rendering the key dimerization step difficult to monitor by TLC and the purification of the product problematic. As an alternative, we attempted selective benzoylation of the 2- and 2′-hydroxyl

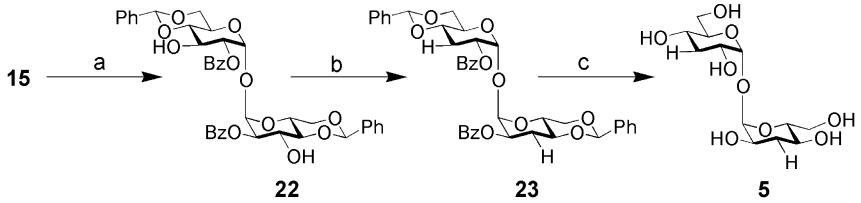
groups of dibenzylidene acetal **15** using the mild acylating reagent, benzoyl imidazole, which has been reported to give the desired 3,3′-diol **22** (Scheme 6) as the major product in 75% yield.[38] In our hands, we obtained a 4:1 mixture of **22** and the 2,3′-dibenzoate regioisomer, which were inseparable by silica gel chromatography. However, treating **15** with 2 equiv of another mild benzoylating reagent, benzoyl cyanide,[39] provided pure **22** with virtually no other contaminating isomers (45% isolated yield plus starting material) (Scheme 6). Subsequent conversion of diol **22** to the respective thionocarbonate, followed by radical deoxygenation and deprotection, yielded desired analog **5**.

Synthesis of the 2,2′-dideoxy analog from intermediate **15** utilizes the regioselective reductive etherification method as described by Wang et al. (Scheme 7),[40] and excellent regioselectivity was observed for this reaction. However, in our hands, **24** was obtained in 38% yield for this three-step procedure, but we observed a significant amount of unreacted TMS–ether starting material during the reductive etherification reaction. Further addition of reagents led to nonspecific etherification of both the C2- and C3-hydroxyl groups. Nonetheless, diol **24** was obtained and subjected to thionocarbonylation and radical deoxygenation to yield compound **25**. Global deprotection gave the 2,2′-dideoxytrehalose analog **6**.

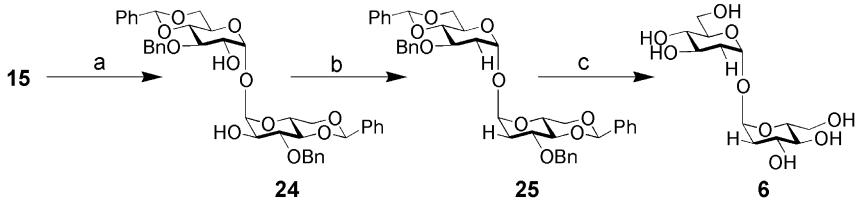
2.4. Synthesis of 3-monodeoxy (9) and 2-monodeoxy (10) analogs

The synthesis of the two remaining monodeoxy analogs **9** and **10** utilizes the known tricyclohexylidene acetal of trehalose **26** as a common intermediate (Scheme 8).[41,42] The regioselective palmitoylation of the 2-hydroxyl group in compound **26** was achieved previously using DCC–DMAP–palmitic acid in the synthesis of trehalose-2-palmitate.[41] Consistent with this previous finding, benzoylation using benzoic acid–DCC–DMAP provided the desired 2′-*O*-benzoate ester **27** in 80% isolated yield (Scheme 8). As a side note, benzoylation using benzoyl chloride (instead of benzoic acid) and DCC–DMAP produced a 1:1 mixture of the 2′- and 3′-monobenzoyl derivatives. The poor regioselectivity observed presumably was due to benzoyl chloride being a more reactive acylating reagent than DCC-activated benzoic acid. With the 2′-hydroxyl protected, conversion of the 3′-hydroxyl to the corresponding thionocarbonate, followed by radical deoxygenation and deprotection, yielded the 3′-deoxy analog **9** (Scheme 8).

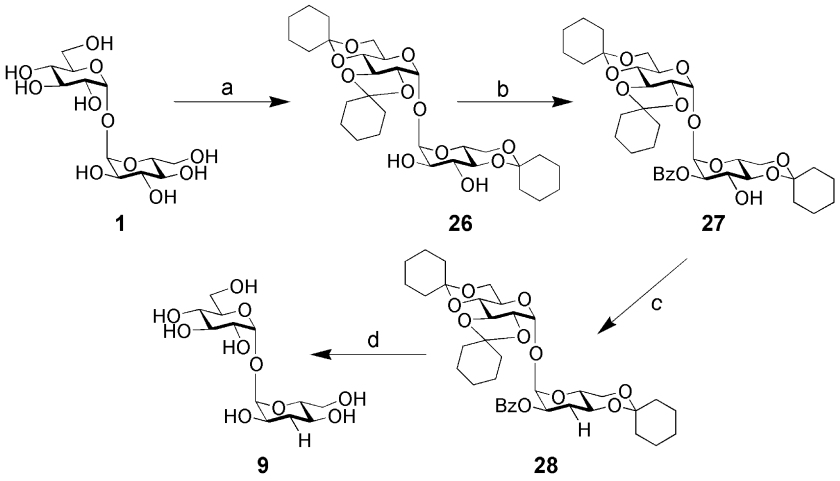
To access the 2′-monodeoxytrehalose analog **10**, we first attempted direct, selective thionocarbonylation of the 2′-hydroxyl of **26** (Scheme 8). Unfortunately, no selectivity between the 2′- and 3′-hydroxyl groups was observed, presumably due to the high reactivity of the thionochloroformate. We then focused on a suitable protecting group for the 3′-hydroxyl of compound **27**, which would have to be installed under relatively neutral conditions due to the base-sensitive 2′-*O*-benzoyl group and the highly acid-sensitive *trans*-fused 2,3-*O*-cyclohexylidene. A few candidates were surveyed, including the introduction of a benzyl group at the 3′-hydroxyl using benzyl trichloroacetimidate and a catalytic amount of trifluoromethanesulfonic acid (TfOH) or benzyl bromide–Ag_2O. The first set of conditions led to degradation of the starting material, even when using a catalytic amount (5 mol %) of acid. The Ag_2O-catalyzed benzylation was very sluggish, with little product formation (by TLC) even after heating at 60 °C for three days. Eventually, we successfully installed a methoxy-



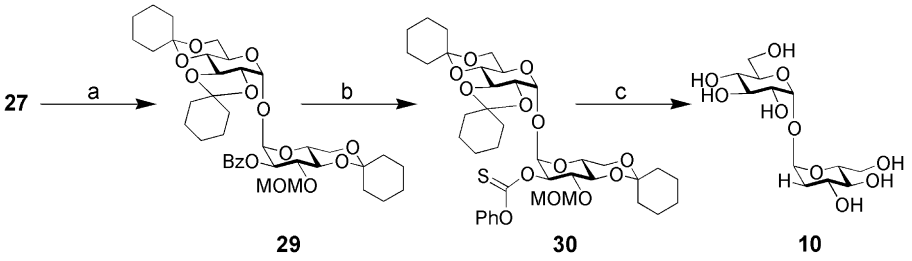
Scheme 6. Reagents: (a) BzCN, Et_3N, MeCN (45%); (b) (i) PTC-Cl, DMAP, MeCN; (ii) Bu_3SnH, AIBN (cat.), PhMe (67% over two steps); (c) (i) NaOMe, MeOH; (ii) TFA–MeOH (14% over two steps).



Scheme 7. Reagents: (a) (i) TMS–Cl, Et_3N, MeCN; (ii) Et_3SiH, PhCHO, TMSOTf (cat.), CH_2Cl_2; (iii) 1.0 M TBAF in THF (38% over three steps); (b) (i) PTC-Cl, DMAP, MeCN; (ii) Bu_3SnH, AIBN (cat.), PhMe (51% over two steps); (c) H_2, $Pd(OH)_2/C$, AcOH–MeOH (91%).



Scheme 8. Reagents: (a) Cyclohexanone dimethyl ketal, CSA (cat.), DMF (36%); (b) BzOH, DCC, DMAP, CH_2Cl_2 (80%); (c) (i) PTC-Cl, DMAP, MeCN; (ii) Bu_3SnH, AIBN (cat.), PhMe (55% over two steps); (c) (i) NaOMe, MeOH; (ii) 10% aq HCl–THF (60% over two steps).



Scheme 9. Reagents: (a) MOM-Cl, TBAI, DIPEA (90%); (b) (i) NaOMe, MeOH; (ii) PTC-Cl, DMAP, MeCN (75% over two steps); (c) (i) Bu_3SnH, AIBN (cat.), PhMe; (ii) 10% aq HCl–THF (40% over two steps).

methyl (MOM) group on the 3′-hydroxyl to afford compound **29** (Scheme 9). Following the standard procedure for installing MOM groups using MOM-Cl (in large excess) in Hünig's base,[43] the reaction was extremely slow even when heated at 60 °C. The addition of tetrabutylammonium iodide (TBAI), however, greatly accelerated the reaction rate. Greater than 90% conversion of **27** to **29** could be obtained in 30 min using stoichiometric amounts of TBAI at 60 °C. Removal of the 2′-benzoyl group of **29** under Zemplén conditions exposed the 2′-hydroxyl group, which was subsequently converted to the corresponding thionocarbonate (**30**). Radical deoxygenation of **30**, followed by acidic cleavage of the cyclohexylidene and the MOM groups, yielded 2′-monodeoxytrehalose **10**. The moderate yields of the last two steps reflect a partial loss of the fully deprotected, highly polar disaccharide product during purification.

2.5. Complete assignment of ^1H and ^{13}C NMR resonances for the mono- and dideoxytrehaloses

The assignment of all the ^1H and ^{13}C resonances for each of the disaccharides proved to be nontrivial, due to severe overlap of signals in the ^1H NMR spectra. A combination of two-dimensional (2D) NMR techniques including $\{^1H,^1H\}$ COSY, $\{^1H,^1H\}$ TOCSY, $\{^1H,^{13}C\}$ HSQC and $\{^1H,^{13}C\}$ HMBC was employed to accomplish this task. The strategies utilized for assignment followed those outlined in the carbohydrate NMR literature,[44,45] with a few modifications tailored to the spectral complexity of the compounds at hand.

The complete assignment of the ^1H and ^{13}C signals of 2′-monodeoxytrehalose **10** is discussed here as an example. Figure 3a shows the 1D ^1H NMR spectrum of **10**. Taking into consideration the characteristic patterns for ^1H signals from α-D-glucopyranosyl rings, most of the non-overlapping ^1H signals (including the H1–H2, H4–H5, and H1′–H2′ax–H2′eq–H3′–H4′ connectivity trails) were assigned by 2D $\{^1H,^1H\}$ COSY and TOCSY. (2D NMR spectra are available in the Supplementary data). The ^{13}C signals corresponding to the aforementioned hydrogens then were assigned via 2D one-bond $\{^1H,^{13}C\}$ correlation (HSQC). However, the region of the ^1H NMR spectrum between 3.6 and 3.8 ppm is very crowded. Unambiguous assignment of all signals based solely on analysis of their splitting patterns was not possible in this area, due to higher order effects even at 800 MHz.

Sorting of ^1H signals using ^{13}C chemical shifts (by HSQC) helped tremendously, due to the larger chemical

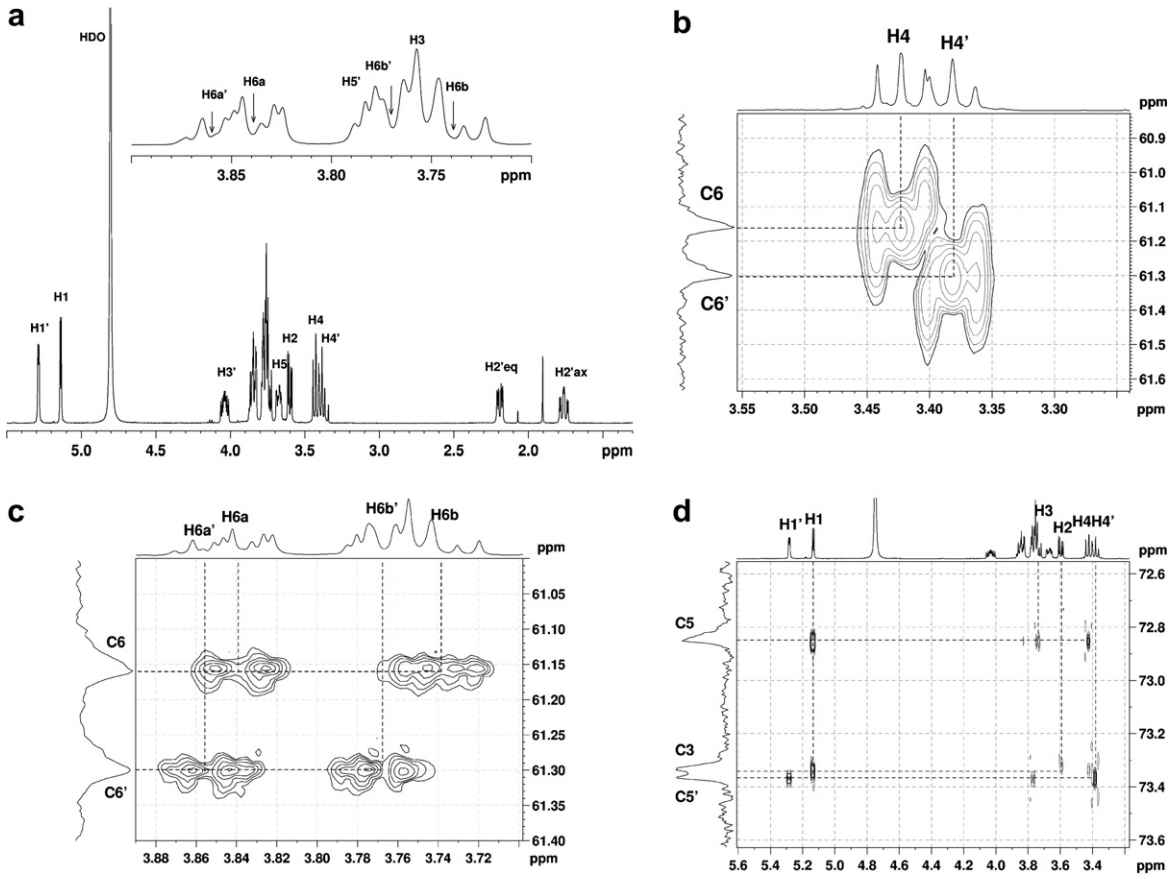


Figure 3. (a) 1D ^{1}H NMR spectrum of 2′-deoxytrehalose (**10**) in D_2O recorded at 500 MHz. (b) 2D {^{1}H,^{13}C} HMBC spectrum of **10**. Cross-peaks resulting from long-range coupling between H4′ and C6′, and between H4 and C6, allowed the assignment of these two carbon resonances. (c) 2D {^{1}H,^{13}C} HSQC spectrum of **10** showing the assignment of H6a′/H6b′ and H6a/H6b via their correlations to the C6′ and C6 signals, respectively. (d) 2D {^{1}H,^{13}C} HMBC spectrum of **10**, with a small sweep width (10 ppm) in the ^{13}C dimension. Cross-peaks resulting from H1′–C5′, H1–C3, H4′–C5′ and H2–C3 long-range couplings allowed the definitive assignment of C5′ and C3, the signals of which are just 0.02 ppm apart.

shift dispersion of the ^{13}C signals. Six overlapping proton resonances corresponding to H3, H5′, H6a, H6b, H6a′ and H6b′ occur within this 0.2 ppm window. The two methylenic C6 carbons were readily identified via a ^{13}C DEPT-135 experiment. Assignment of these two C6 carbons was achieved by analyzing the 2D {^{1}H,^{13}C} HMBC spectrum of **10** (Fig. 3b). With the H4 and H4′ signals assigned (see above), the $^{3}J_{CH}$ cross-peak between H4′ and C6′, and the cross-peak between H4 and C6 allowed the unambiguous assignment of C6 and C6′. Consequently, the multiplets at 3.82–3.88 ppm in the ^{1}H spectrum were attributed to two H6 protons (H6a and H6a′) based on cross-peaks between these signals and C6 or C6′, respectively, in the HSQC spectrum (Fig. 3c). Protons H6b and H6b′ were assigned in an analogous fashion, by virtue of their correlations to C6 and C6′ (Fig. 3c).

As for the remaining two signals, not only were H3 and H5′ found to overlap in the ^{1}H spectrum, but their corresponding ^{13}C resonances also were very close to each other, differing by only 0.02 ppm. Definitive assignment of C3 and C5′ was based on the C3–H1 and C5′–H4′ cross-peaks in the HMBC spectrum (Fig. 3d). Following the assignment of C3 and C5′, the exact chemical shifts of the H3 and H5′ resonances from the HSQC spectrum were obtained.

Tables 1–3 summarize the ^{1}H and ^{13}C chemical shifts, as well as the J_{HH} coupling constants, for trehalose and the mono- and dideoxygenated analogs synthesized in this study. The ^{1}H and ^{13}C chemical shifts at the sites of deoxygenation in the deoxytrehaloses are shifted upfield relative to those in unmodified trehalose, consistent with the removal of the deshielding effects exerted by the electronegative oxygen atom (Tables 1 and 2). The carbons adjacent to the deoxygenation sites are also influenced by deoxygenation, as revealed by a slight upfield shift of ~5 ppm (Table 2). Furthermore, in the monodeoxygenated trehaloses, the ^{1}H and ^{13}C chemical shifts in the unmodified glucose rings are almost identical to those observed for trehalose, whereas the values for the deoxygenated rings are remarkably similar to those of the corresponding dideoxygenated analog. This observation indicates that the effects imparted by deoxygenation are localized in the modified glucose ring (Tables 1 and 2). Severe spectral overlap hampered the

Table 1. ^{1}H chemical shifts (in ppm) of α,α-trehalose (**1**) and deoxytrehalose analogs **3**–**10**

	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	H-1′	H-2′	H-3′	H-4′	H-5′	H-6a′	H-6b′
α,α-Trehalose (**1**)	5.18	3.64	3.84	3.43	3.81	3.85	3.75	—	—	—	—	—	—	—
6-Dideoxy (**3**)	—	—	—	—	—	—	—	5.09	3.64	3.77	3.18	3.86	1.26	—
4-Dideoxy (**4**)	—	—	—	—	—	—	—	5.20	3.55	4.08	2.00 (eq) 1.46 (ax)	4.08	3.66	3.58
3-Dideoxy (**5**)	—	—	—	—	—	—	—	5.13	3.88	2.20 (eq) 1.90 (ax)	3.65	3.67	3.84	3.71
2-Dideoxy (**6**)	—	—	—	—	—	—	—	5.25	2.16 (eq) 1.73 (ax)	3.97	3.38	3.65	3.84	3.76
6-Monodeoxy (**7**)	5.15	3.63	3.83	3.43	3.80	3.84	3.75	5.12	3.65	3.78	3.18	3.88	1.26	—
4-Monodeoxy (**8**)	5.18	3.63	3.84	3.43	3.83	3.86	3.75	5.21	3.56	4.09	2.01 (eq) 1.47 (ax)	4.09	3.67	3.58
3-Monodeoxy (**9**)	5.22	3.64	3.70	3.45	3.78	3.84	3.74	5.08	3.86	2.19 (eq) 1.88 (ax)	3.65	3.69	3.83	3.70
2-Monodeoxy (**10**)	5.14	3.60	3.76	3.42	3.67	3.84	3.74	5.29	2.19 (eq) 1.76 (ax)	4.04	3.38	3.76	3.86	3.77

Prime denotes deoxygenated glucose ring.

Table 2. ^{13}C chemical shifts (in ppm) of α,α-trehalose (**1**) and deoxytrehalose analogs **3**–**10**

	C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
α,α-Trehalose (**1**)	93.84	71.66	73.14	70.32	72.76	61.16	—	—	—	—	—	—
6-Dideoxy (**3**)	—	—	—	—	—	—	93.91	71.95	72.94	75.86	68.84	17.21
4-Dideoxy (**4**)	—	—	—	—	—	—	94.33	73.52	69.77	34.82	67.37	64.28
3-Dideoxy (**5**)	—	—	—	—	—	—	92.67	66.95	34.50	64.91	73.40	61.25
2-Dideoxy (**6**)	—	—	—	—	—	—	93.13	36.97	68.74	71.61	73.39	61.30
6-Monodeoxy (**7**)	93.96	71.70	73.18	70.34	72.79	61.15	93.83	71.94	72.93	75.85	68.84	17.20
4-Monodeoxy (**8**)	92.90	70.92	72.38	69.53	71.90	60.35	93.64	72.64	69.04	34.01	66.49	63.44
3-Monodeoxy (**9**)	93.76	71.72	73.16	70.29	72.65	61.15	92.67	66.78	34.42	64.83	73.34	61.25
2-Monodeoxy (**10**)	93.89	71.67	73.33	70.35	72.87	61.14	92.97	37.02	68.60	71.67	73.36	61.30

Prime denotes deoxygenated glucose ring.

Table 3. J_{HH} coupling constants (in Hz) of α,α-trehalose (**1**) and deoxytrehalose analogs **3**–**10**

Compound	^{1}H Coupling constants, J (Hz)
α,α-Trehalose (**1**)	3.8 ($J_{1,2}$), 9.8 ($J_{2,3}$), 9.8 ($J_{3,4}$), 9.8 ($J_{4,5}$), 2.0 ($J_{5,6a}$), 5.1 ($J_{5,6b}$), 11.9 ($J_{6a,6b}$)
6,6′-Dideoxy (**3**)	3.8 ($J_{1',2'}$), 9.6 ($J_{2',3'}$), 9.6 ($J_{3',4'}$), 9.6 ($J_{4',5'}$), 6.2 ($J_{5',6'}$)
4,4′-Dideoxy (**4**)	3.8 ($J_{1',2'}$), 9.8 ($J_{2',3'}$), 12.7 ($J_{3',4ax'}$), 12.7 ($J_{4eq',4ax'}$), 12.7 ($J_{4ax',5'}$), 2.1[a] ($J_{3',4eq'}$) or ($J_{4eq',5'}$), 5.2[a] ($J_{3',4eq'}$) or ($J_{4eq',5'}$)
3,3′-Dideoxy (**5**)	3.4($J_{1',2'}$), 12.0 ($J_{6a',6b'}$)[b]
2,2′-Dideoxy (**6**)	3.7($J_{1',2ax'}$), 1.0 ($J_{1',2eq'}$), 11.8 ($J_{2ax',3'}$), 5.1 ($J_{2eq',3'}$), 13.4 ($J_{2ax',2eq'}$), 9.6 ($J_{3',4'}$), 9.6 ($J_{4',5'}$), 2.3 ($J_{5',6a'}$), 5.5 ($J_{5',6b'}$), 12.2 ($J_{6a',6b'}$)
6′-Monodeoxy (**7**)	3.8 ($J_{1,2}$), 9.8 ($J_{2,3}$), 9.8 ($J_{3,4}$), 9.8 ($J_{4,5}$), 3.8 ($J_{1',2'}$), 9.8 ($J_{2',3'}$), 9.8 ($J_{3',4'}$), 9.8 ($J_{4',5'}$), 6.2 ($J_{5',6'}$)
4′-Monodeoxy (**8**)	3.7 ($J_{1,2}$), 9.6 ($J_{2,3}$), 9.6 ($J_{3,4}$), 9.6 ($J_{4,5}$), 5.3 ($J_{5,6b}$), 12.1 ($J_{6a,6b}$), 3.8 ($J_{1',2'}$), 9.8 ($J_{2',3'}$), 12.7 ($J_{3',4ax'}$), 12.7 ($J_{4ax',4eq'}$), 12.7 ($J_{4ax',5'}$), 2.0[c] ($J_{3',4eq'}$) or ($J_{4eq',5'}$), 5.5[c] ($J_{3',4eq'}$) or ($J_{4eq',5'}$), 3.1 ($J_{5',6a'}$), 6.4 ($J_{5',6b'}$), 12.1 ($J_{6a',6b'}$)
3′-Monodeoxy (**9**)	3.8 ($J_{1,2}$), 9.7 ($J_{2,3}$), 9.7 ($J_{3,4}$), 3.6 ($J_{1',2'}$), 4.5 ($J_{2',3eq'}$), 11.2 ($J_{2',3ax'}$), 11.2 ($J_{3eq',3ax'}$), 4.5 ($J_{3eq',4'}$), 11.2 ($J_{3ax',4'}$)
2-Monodeoxy (**10**)	3.8 ($J_{1,2}$), 9.7 ($J_{2,3}$), 9.7 ($J_{3,4}$), 9.7 ($J_{4,5}$), 2.2 ($J_{5,6a}$), 5.5 ($J_{5,6b}$), 3.8 ($J_{1',2ax'}$), 1.0 ($J_{1',2eq'}$), 5.2 ($J_{2eq',3'}$), 11.8 ($J_{2ax',3'}$), 5.1 ($J_{2eq',3'}$), 13.3 ($J_{2eq',2ax'}$), 9.6 ($J_{3',4'}$)

Prime denotes deoxygenated glucose ring.
Some coupling constants cannot be measured due to overlap of signals in the spectrum.
[a] H-3′ and H-5′ have the same chemical shift values; therefore, the coupling between H-4eq′ and these two protons cannot be distinguished.
[b] Due to spectral overlap and the complex appearance of H-3ax′ and H-3eq′, only two coupling constants can be measured for this compound. This observation is consistent with previously reported spectral data in Ref. 37.
[c] H-3′ and H-5′ have the same chemical shift values; therefore, the coupling between H-4eq′ and these two protons cannot be distinguished.

measurement of all ^{1}H–^{1}H coupling constants; however, the coupling constants measured for the deoxytrehaloses are very similar to those measured for trehalose (Table 3). Presumably, the removal of one or two hydroxyl groups does not affect the predominant solution conformations of the disaccharide to a large extent.

3. Conclusions

This study represents the first systematic synthesis of a panel of mono- and dideoxygenated α,α-trehalose analogs, four of which (**6**, **8**, **9** and **10**) have not been previously reported. NMR experiments of these compounds have furthermore allowed for the full assignment of all ^{1}H and ^{13}C resonances for each compound. The synthetic trehalose analogs will be used in future experiments to characterize the substrate binding interactions in the active site of mycobacterial sulfotransferase Stf0.

4. Experimental

4.1. General materials and methods

All chemical reagents were of analytical grade, obtained from commercial suppliers and used without further purification. Flash chromatography was performed using silica gel. Analytical TLC was performed on silica gel plates, and visualized by charring with ethanolic H_2SO_4 or ceric ammonium molybdate (CAM) stain, and/or by absorbance of UV light at 254 nm. All air- and moisture-sensitive reactions were performed under an atmosphere of N_2. CH_2Cl_2, MeCN and toluene were dried by distilling from calcium hydride. THF was distilled from sodium metal/benzophenone, dry DMF was obtained from commercial suppliers and MeOH was dried over Mg°. All organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure using a rotary evaporator.

Low and high-resolution fast-atom bombardment (FAB) and low-resolution electrospray ionization (ESI) mass spectra were obtained at the UC Berkeley Mass Spectrometry Laboratory. High-resolution electrospray ionization mass spectra were obtained at the Scripps Mass Spectrometry Laboratory. Elemental analyses were obtained at the UC Berkeley Microanalytical Laboratory.

4.2. NMR spectroscopy

Prior to NMR studies, hydroxyl hydrogens in the unprotected disaccharides were exchanged with deuterium by repeated dissolution in 99.9% D_2O with intermittent lyophilization. Each of the deuterium-exchanged saccharides (20–40 μmol) was dissolved in 0.6 mL of D_2O (99.96% D; Cambridge Isotope Labs, Andover, MA) and subjected to ^{1}H and ^{13}C NMR analysis. The majority of 1D and 2D NMR experiments, unless otherwise noted, were performed at 27 °C on a Bruker Avance 500 spectrometer, interfaced with a Hewlett–Packard workstation running XWinNMR 3.5 under Linux 7.1, using a 5-mm TBI (triple-channel, broadband, inverse) probe head with a *z*-gradient coil. 1D ^{1}H NMR spectra of trehalose and its deoxygenated analogs were also recorded at 800 MHz and ambient temperature, using a Bruker Avance 800 spectrometer equipped with a 5-mm TCI cryoprobe. 1D ^{13}C and DEPT experiments were performed at ambient temperature on a Bruker DRX-500 spectrometer, equipped with a 5-mm broadband-observe probe head. Data were processed off-line using Bruker's TopSpin for MS Windows 2000/XP software (version 1.3) (Bruker BioSpin, Billerica, MA). ^{1}H chemical shifts are reported relative to 2,2-dimethyl-silapentane-5-sulfonic acid (DSS) (but were actually measured by reference to internal acetone at δ 2.225 ppm in D_2O) with an accuracy of 0.003 ppm. ^{13}C chemical shifts are referenced to the methyl signal of acetone in D_2O (δ CH_3 30.89 ppm, relative to DSS).

2D {^{1}H,^{1}H} gs-COSY data sets were collected in magnitude mode;[46–48] 2D {^{1}H,^{1}H} TOCSY[49,50] data sets were collected in phase-sensitive mode using the TPPI method.[51] The TOCSY pulse program contained a 30–120 ms MLEV-17 spin-lock pulse train.[52] 2D {^{1}H,^{13}C} HSQC[53,54] and HMBC[55,56] experiments were performed with pulsed field gradients for coherence selection, using echo–antiecho phase-sensitive detection. The 2D data were typically processed with shifted squared sine bell functions applied in both the t_2 and the t_1 dimensions, along with zero-filling in the t_1 dimension.

4.3. Syntheses

The known compounds **15**,[28] **16**,[24] **24**[40] and **26**[42] were prepared according to literature procedures.

4.3.1. 6,6′-Di-*O*-*tert*-butyldimethylsilyl-2,3,4,2′,3′,4′-hexa-*O*-benzoyl-α,α-D-trehalose (11). To a solution of anhydrous α,α′-trehalose (5.0 g, 15 mmol) in 60 mL of dry pyridine was added *tert*-butyldimethylchlorosilane (4.8 g, 32 mmol) at 0 °C (ice-water bath). A catalytic amount of DMAP (0.5 g, 5 mmol) was added, and the resulting pale-yellow reaction mixture was stirred overnight, after which TLC analysis indicated complete consumption of starting material. The reaction mixture was cooled to 0 °C, and benzoyl chloride (20.3 mL, 175 mmol) was added. The reaction mixture was warmed to rt and stirred overnight, after which the reaction was complete as indicated by TLC analysis. The reaction mixture was diluted with CH_2Cl_2, and the precipitate removed by filtration. The filtrate was washed with H_2O and brine. The crude product, a yellow oil, was purified by flash column chromatography (3:7 EtOAc–hexanes) to yield 16 g (92%) of **11** as white amorphous solids (R_f 0.45, 2:8 EtOAc–hexanes). ^{1}H NMR (500 MHz, $CDCl_3$): δ −0.15 (d, 12H, $J = 7.5$), 0.79 (s, 18H), 3.10–3.17 (m, 4H), 4.00 (d, 2H, $J = 10.0$), 5.40 (dd, 2H, $J = 10.0$, 4.0), 5.64–5.68 (m, 4H), 6.20 (app. t, 2H, $J = 10.0$), 7.32–7.56 (m, 18H),

7.80 (d, 4H, $J = 7.5$), 7.94 (d, 4H, $J = 7.0$), 8.14 (d, 4H, $J = 7.5$); ^{13}C NMR (125 MHz, $CDCl_3$): δ −5.46, −5.47, 18.5, 26.0, 60.9, 68.2, 70.9, 71.2, 71.8, 93.1, 128.49, 128.52, 128.9, 129.1, 129.5, 129.6, 129.98, 130.02, 130.2, 133.2, 133.4, 133.7, 164.8, 165.5, 165.9. LRFABMS (m/z): $[M+Li]^+$ calcd for $C_{66}H_{74}O_{17}Si_2Li$, 1201.5; found, 1201.4. Anal. Calcd for $C_{66}H_{74}O_{17}Si_2$: C, 66.31; H, 6.24. Found: C, 66.06; H, 6.12.

4.3.2. 2,3,4,2′,3′,4′-Hexa-*O*-benzoyl-α,α-D-trehalose (12). Compound **11** (3.42 g, 2.86 mmol) was dissolved in dry THF (50 mL) with 10% (v/v) AcOH under N_2. A 1.0 M solution of TBAF in THF (12 mL, 12 mmol) was added, and the reaction mixture was stirred and monitored by TLC. After ∼24 h, the reaction appeared to be complete, and the solvent was removed. The crude product was purified by flash chromatography (1:1 EtOAc–hexanes) to yield 2.0 g of **12** (72%) as a white solid (R_f 0.3, 1:1 EtOAc–hexanes). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.43 (app. t, 2H, $J = 7.0$), 2.83–2.87 (m, 2H), 3.04–3.08 (m, 2H), 3.83 (app. d, 2H, $J = 10.0$), 5.32 (dd, 2H, $J = 10.5$, 4.0), 5.42 (app. t, 2H, $J = 10.0$), 5.64 (d, 2H, $J = 4.0$), 6.24 (app. t, 2H, $J = 10.0$), 7.18–7.39 (m, 16H), 7.50–7.53 (m, 2H), 7.83–7.86 (m, 8H), 7.98 (d, 4H, $J = 7.5$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 60.5, 68.9, 70.1, 70.66, 71.70, 93.4, 128.6, 128.7, 128.8, 129.3, 129.9, 130.0, 130.3, 133.5, 133.9, 134.0, 165.5, 165.8, 166.4. LRFABMS (m/z): $[M+H]^+$ calcd for $C_{64}H_{46}O_{17}$, 966.3; found, 967.6. Anal. Calcd for $C_{64}H_{46}O_{17}$: C, 67.08; H, 4.80. Found: C, 66.87; H, 4.94.

4.3.3. 6,6′-Di-*O*-phenoxythiocarbonyl-2,3,4,2′,3′,4′-hexa-*O*-benzoyl-α,α-D-trehalose (13). Diol **12** (0.84 g, 0.87 mmol) and DMAP (0.43 g, 3.5 mmol) were dissolved in 40 mL of dry MeCN. Phenyl thionochloroformate (PTC-Cl) (260 μL, 1.9 mmol) was added to this solution, causing the solution to become bright yellow. The reaction mixture was stirred at rt overnight, after which the bright yellow color had faded and TLC analysis indicated complete consumption of starting material. The solvent was removed, and the resulting pale-yellow residue was subjected to flash chromatography (3:7 EtOAc–hexanes) to yield 0.70 g (65%) of **13** as a white solid (R_f 0.26, 2:8 EtOAc–hexanes). ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.98 (dd, 2H, $J = 12.0$, 2.5), 4.07 (dd, 2H, $J = 12.0$, 4.0), 4.28–4.30 (m, 2H), 5.47 (dd, 2H, $J = 10.5$, 4.0), 5.65 (app. t, 2H, $J = 10.0$), 5.75 (d, 2H, $J = 4.0$), 6.28 (app. t, 2H, $J = 10.0$), 7.10 (app. d, 4H, $J = 8.0$), 7.26–7.47 (m, 22H), 7.56 (app. t, 2H, $J = 7.5$), 7.82 (app. d, 4H, $J = 7.5$), 7.94 (app. d, 4H, $J = 7.5$), 8.13 (app. d, 4H, $J = 7.5$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 68.4, 68.5, 70.3, 70.4, 71.5, 93.4, 122.2, 126.8, 128.6, 128.7, 128.9, 129.1, 129.3, 129.7, 130.0, 130.2, 130.2, 133.5, 133.8, 134.1, 153.7, 165.2, 165.5, 165.8, 194.8. HRFABMS (m/z): $[M+Li]^+$ calcd for $C_{68}H_{54}O_{19}S_2Li$, 1245.2861; found, 1245.2875.

4.3.4. 6,6′-Dideoxy-α,α-D-trehalose (3). Compound **13** (0.60 g, 0.48 mmol) and azobisisobutylonitrile (AIBN) (0.032 g, 0.19 mmol) were dissolved in 10 mL of dry toluene. Tributyltin hydride (Bu_3SnH) (1.4 mL, 4.8 mmol) was added in one portion. The solution was heated at reflux for 30 min, after which TLC analysis indicated complete consumption of the starting material. The reaction mixture was cooled to rt, and PhMe was removed under reduced pressure. The resulting residue was redissolved in 40 mL of MeCN and washed with pentane to remove excess Bu_3SnH and other byproducts. Following removal of MeCN, the crude product was resuspended in 20 mL of dry MeOH and 0.18 mL of a 1.0 M solution of NaOMe in MeOH was added. After stirring at rt for 12 h, TLC analysis indicated the presence of a highly polar compound (R_f 0.52, 7:2:1 EtOAc–MeOH–H_2O). The reaction mixture was neutralized by addition of Dowex 50X-W8 resin (H^+ form). After removal of the resin by filtration, the filtrate was concentrated to a clear oil as crude product. The crude product was purified by flash chromatography (7:2:1 EtOAc–MeOH–H_2O) to yield a white residue. Residual water was removed by lyophilization to yield 20 mg (36%) of **3** as a white, fluffy solid. NMR data are summarized in Tables 1–3. HRFABMS (m/z): $[M+Na]^+$ calcd for $C_{12}H_{22}O_9Na$, 333.1162; found, 333.1164.

4.3.5. 2,3,4,6,2′,3′,4′-Hepta-*O*-benzoyl-6′-*O*-phenoxythiocarbonyl-α,α-D-trehalose (14). Diol **12** (1.59 g, 1.64 mmol), benzoyl chloride (0.21 mL, 1.8 mmol) and DMAP (∼10 mg) were dissolved in 10 mL of dry pyridine. The reaction mixture was stirred at rt and monitored by TLC. After ∼12 h, two new, less polar spots in ∼1:1 ratio were observed, corresponding to the mono- and dibenzoylated products, as well as some unreacted starting material. The reaction mixture was concentrated and subjected to flash chromatography (2:8 EtOAc–hexanes). The partially purified monobenzoylated product (0.198 g, 0.185 mmol) was treated with PTC-Cl (30 μL, 0.22 mmol) and DMAP (0.045 g, 0.37 mmol) in 10 mL of dry MeCN. After stirring overnight, the reaction mixture was concentrated, resuspended in EtOAc (50 mL), and the organic layer was washed with H_2O and brine. The crude product was purified by flash chromatography (3:7 EtOAc–hexanes) to yield 140 mg (63%) of **14** as a white foam (R_f 0.69, 3:7 EtOAc–hexanes). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.90 (dd, 1H, $J = 12.4$, 4.6), 4.02–4.10 (m, 2H), 4.11 (dd, 1H, $J = 12.4$, 4.0), 4.32–4.39 (m, 2H), 5.51–5.59 (m, 2H), 5.68 (app. t, 1H, $J = 9.8$), 5.71 (app. t, 1H, $J = 9.8$), 5.76 (d, 1H, $J = 3.8$), 5.79 (d, 1H, $J = 3.8$), 6.31 (app. dt, 2H, $J = 9.8$, 2.0), 7.08–7.14 (m, 2H), 7.25–7.51 (m, 21H), 7.53–7.61 (m, 3H), 7.85–7.90 (m,

4H), 8.07–8.11 (m, 6H), 8.12–8.17 (m, 4H); ^{13}C DEPT135 NMR (100 MHz, $CDCl_3$): δ 62.1 (C6, CH_2), 68.4, 68.6, 68.8, 68.9, 70.4 (C6′, CH_2), 70.5, 70.6, 71.4, 71.6, 93.0, 122.2, 126.8, 128.7, 129.0, 129.1, 129.8, 130.0, 130.16, 130.19, 130.21, 133.4, 133.5, 133.8, 134.1, 134.2. HRFABMS (*m*/*z*): $[M+Li]^+$ calcd for $C_{68}H_{54}O_{19}SLi$, 1213.3140; found, 1213.3141.

4.3.6. 6-Deoxy-α,α-D-trehalose (7). Compound **14** (120 mg, 0.099 mmol) and AIBN (3.0 mg, 0.02 mmol) were dissolved in 10 mL of dry toluene. Bu_3SnH (0.14 mL, 0.50 mmol) was added in one portion. The reaction mixture was heated at reflux for 30 min, after which TLC analysis indicated complete consumption of starting material. The reaction mixture was cooled to rt, and toluene was removed under reduced pressure. The resulting residue was redissolved in 40 mL of MeCN and washed with pentane (3 × 20 mL) to remove excess Bu_3SnH and other byproducts. The crude product was partially purified by silica gel flash chromatography (2:8 EtOAc–hexanes) to yield 90 mg (87%) of a white foam. This product (73 mg) was resuspended in 5 mL of anhyd MeOH, and 30 μL of a 1.0 M solution of NaOMe in MeOH was added. After stirring at rt for 12 h, TLC analysis indicated the presence of a highly polar compound (R_f 0.52 in 7:2:1 EtOAc–MeOH–H_2O). The reaction mixture was neutralized by addition of Dowex 50X-W8 resin (H^+ form). After removal of the resin by filtration, the filtrate was concentrated to a clear oil as crude product. The crude product was purified by flash chromatography (7:2:1 EtOAc–MeOH–H_2O) to yield a white residue. Residual water was removed by lyophilization to yield 16 mg (69%) of **7** as a white, fluffy solid. NMR data are summarized in Tables 1–3. HRESIMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{12}H_{22}O_{10}Na$, 349.1105; found, 349.1105.

4.3.7. 2,3,6,2′,3′,6′-Hexa-*O*-benzyl-α,α-D-trehalose (17a). Compound **16** (8.5 g, 9.7 mmol) was dissolved in 80 mL of dry THF, to which 3 Å molecular sieves (1 g) and $NaCNBH_3$ (1.40 g, 22.3 mmol) were added. The resulting pale-yellow suspension was stirred at rt for 10 min. A solution of 1.65 mL (22.3 mmol) of trifluoromethanesulfonic acid (TfOH) in 5 mL of THF was added dropwise to the reaction mixture, resulting in significant gas evolution. Addition of TfOH was discontinued when no more bubbling was observed. The reaction progressed very slowly as monitored by TLC, even after being stirred for several hours. Additional $NaCNBH_3$ and TfOH (2 equiv each) were added every 2 h, but the reaction had not progressed any further after addition of an extra 4 equiv of reagents. At this point, the reaction mixture was filtered through Celite to remove the molecular sieves and salt, concentrated and redissolved in CH_2Cl_2. The organic layer was washed with H_2O and brine. After removal of solvent, the crude product (yellow oil) was partially purified by flash chromatography (2:8 EtOAc–hexanes) to yield 4 g of a 1:1 mixture of **17a** (R_f 0.56, 3:7 EtOAc–hexanes) and monobenzylidene **17b** (R_f 0.62, 3:7 EtOAc–hexanes). The mixture was taken on to the following step without further purification. A small amount (200 mg) of pure **17** was isolated and fully characterized. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.58 (d, 2H, $J = 3.0$), 3.45–3.58 (m, 4H), 3.60 (dd, 2H, $J = 9.5$, 3.8), 3.70 (m, 2H), 3.92 (app. t, 2H, $J = 9.3$), 4.10–4.15 (m, 2H), 4.47 (d, 2H, $J = 12.0$), 4.54, (d, 2H, $J = 12.0$), 4.66 (d, 2H, $J = 12.0$), 4.73 (d, 2H, $J = 12.0$), 4.83 (d, 2H, $J = 11.3$), 5.03 (d, 2H, $J = 11.3$), 5.28 (d, 2H, $J = 3.8$), 7.22–7.44 (m, 30H). ^{13}C DEPT135 NMR (100 MHz, $CDCl_3$): δ 69.1 (C6, CH_2), 69.3, 70.8 (Bn, CH_2), 72.6 (Bn, CH_2), 73.7 (Bn, CH_2), 75.4, 79.1, 81.1, 94.1, 126.3, 127.7, 127.80, 127.83, 127.9, 128.5, 128.5, 128.7. HRFABMS (*m*/*z*): $[M+Li]^+$ calcd for $C_{54}H_{58}O_{11}Li$, 889.4139; found, 889.4141.

4.3.8. 4,4′-Di-*O*-pentafluorophenoxy thiocarbonyl-2,3,6,2′,3′,6′-hexa-*O*-benzyl-α,α-D-trehalose (18a). The 1:1 mixture of **17a** and monobenzylidenated byproduct **17b** was dissolved in 50 mL of dry MeCN, to which DMAP (2.22 g, 18.2 mmol) and pentafluorophenyl thionochloroformate (2.55 mL, 15.9 mmol) were added. The resulting orange solution was stirred at rt for 12 h, by which time a white precipitate had formed. TLC analysis indicated complete conversion of starting materials to two new compounds. The reaction mixture was filtered, and the filtrate was adsorbed onto silica gel and purified by flash chromatography (1:9 to 2:8 EtOAc–hexanes) to yield 1.20 g (9%, two steps) of **18a** as an off-white solid (R_f 0.53, 2:8 EtOAc–hexanes), as well as 1.0 g (11%, two steps) of the monobenzylidenated product **18b** (R_f 0.62, 3:7 EtOAc–hexanes). Note that both compounds decompose slowly upon heating at 45 °C on a rotary evaporator. ^{1}H NMR of **18a** (500 MHz, $CDCl_3$): 3.24 (dd, 2H, $J = 11.1$, 4.0), 3.39 (dd, 2H, $J = 11.1$, 2.0), 3.71 (dd, 2H, $J = 9.7$, 3.4), 4.24 (app. t, 2H, $J = 9.3$), 4.35–4.40 (m, 4H), 4.46 (d, 2H, $J = 11.9$), 4.64, (d, 2H, $J = 11.9$), 4.75 (d, 2H, $J = 11.8$), 4.86 (d, 2H, $J = 11.1$), 4.90 (d, 2H, $J = 11.1$), 5.23, (d, 2H, $J = 3.4$), 5.68 (app. t, 2H, $J = 9.7$), 7.21–7.38 (m, 30H). ^{13}C DEPT135 NMR (125 MHz, $CDCl_3$): δ 67.9 (C6, CH_2), 69.1, 73.6 (C6, Bn), 74.1 (C6, Bn), 75.8 (C6, Bn), 78.7, 79.1, 82.9, 94.0, 127.7, 127.9, 128.0, 128.1, 128.2, 128.49, 128.52, 128.6. HRFABMS (*m*/*z*): $[M+Li]^+$ calcd for $C_{68}H_{56}O_{13}F_{10}S_2Li$, 1341.3163; found, 1341.3176. Anal. Calcd for $C_{68}H_{56}F_{10}O_{13}S_2$: C, 61.17; H, 4.23; S, 4.80. Found: C, 61.03; H, 4.34; S, 4.62.

4.3.9. 4,4′-Dideoxy-α,α-D-trehalose (4). Compound **18a** (1.2 g, 0.90 mmol) was dissolved in 40 mL of dry toluene, to which AIBN (0.059 g, 0.36 mmol) was added,

followed by Bu_3SnH (2.42 mL, 8.99 mmol). The resulting solution was heated at reflux for 1 h, at which point TLC analysis indicated complete consumption of the starting material. The reaction mixture was cooled to rt, concentrated and redissolved in 100 mL of MeCN. The MeCN solution was washed with pentane to remove Sn-containing byproducts, and the combined pentane layers were back-extracted with 50 mL of MeCN. The combined MeCN layers were adsorbed onto silica gel and subjected to flash chromatography (2:8 EtOAc–hexanes) to yield 0.66 g of a clear oil. This oil was dissolved in 10 mL of dry MeOH and subjected to hydrogenolysis using $Pd(OH)_2/C$. (Caution! Extreme fire hazard.) The reaction was monitored by TLC, and hydrogen was bubbled into the solution until all starting material was consumed. The catalyst was removed by filtering through Celite, and the filtrate was adsorbed onto silica gel. Purification by flash chromatography in 7:2:1 EtOAc–MeOH–H_2O yielded a white amorphous solid, from which residual water was removed by lyophilization to yield 102 mg (36%, two steps) of **4** (R_f 0.30, 7:2:1 EtOAc–MeOH–H_2O). NMR data are summarized in Tables 1–3. HRESIMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{12}H_{22}O_9Na$, 333.1156; found, 333.1155.

4.3.10. 2,3,2′,3′-Tetra-*O*-benzyl-4,6-*O*-benzylidene-α,α-D-trehalose (19). Compound **15** (2.0 g, 3.9 mmol) was suspended in 40 mL of dry DMF and cooled to 0 °C in an ice/water bath. NaH (60 wt %, 0.93 g, 32 mmol) was added, and the resulting mixture was stirred for 10 min. Benzyl bromide (2.2 mL, 18 mmol) was added dropwise over 5 min, and the reaction mixture was stirred at rt for 3 h. At this point the reaction appeared to be complete by TLC analysis, and water was added to quench excess NaH. This reaction mixture was diluted with EtOAc and washed with H_2O and brine. The organic layer was dried over Na_2SO_4 and concentrated to yield a pale-yellow residue. This crude product (R_f 0.73, 2:8 EtOAc–hexanes) was redissolved in 20 mL of 9:1 MeOH–CH_2Cl_2, and trifluoroacetic acid (TFA) (2 mL) was added. After 3 h, TLC anaylsis indicated the formation of two new products along with unreacted starting material. The acid was neutralized with solid $NaHCO_3$. Water was added, and the resulting mixture was extracted with EtOAc, which was then washed with water and brine. The organic layer was adsorbed onto silica gel, after which flash chromatography with 1:1 EtOAc–hexanes yielded 1.22 g (40%, two steps) of **19** as a white foam (R_f 0.31, 2:98 MeOH–CH_2Cl_2). ^1H NMR (500 MHz, $CDCl_3$): δ 1.94 (br s, 1H), 2.66 (br s, 1H), 3.58 (dd, 1H, $J = 9.8$, 3.5), 3.61–3.73 (m, 6H), 3.93 (app. t, 1H, $J = 9.2$), 4.05 (m, 1H), 4.14–4.20 (m, 2H), 4.27–4.32 (m, 1H), 4.73–4.76 (m, 3H), 4.83 (app. t, 1H, $J = 12.0$), 4.90 (d, 1H, $J = 11.2$), 5.01 (d, 1H, $J = 11.2$), 5.05 (d, 2H, $J = 11.3$), 5.18 (d, 1H, $J = 3.7$), 5.20 (d, 1H, $J = 3.4$), 5.60 (s, 1), 7.28–7.48 (m, 23H), 7.56 (app. dd, 2H, $J = 8.0$, 1.4). ^{13}C DEPT135 NMR (125 MHz, $CDCl_3$): δ 62.3 (CH_2), 63.2, 69.2 (CH_2), 70.5, 71.6, 73.2 (CH_2), 73.8 (CH_2), 75.48 (CH_2), 75.50, 78.8, 78.9, 79.4, 81.2, 82.5, 94.3, 95.0, 101.4, 126.3, 127.8, 127.89, 127.93, 127.98, 128.02, 128.11, 128.14, 128.4, 128.5, 128.6, 128.73, 128.75, 129.1. HRFABMS (*m*/*z*): $[M+Li]^+$ calcd for $C_{47}H_{50}O_{11}Li$, 797.3513; found, 797.3508.

4.3.11. 2,3,2′,3′-Tetra-*O*-benzyl-4,6-benzylidene-6′-*O*-*tert*-butyldimethylsilyl-α,α-D-trehalose (20). Compound **19** (950 mg, 1.20 mmol) was dissolved in dry CH_2Cl_2 (20 mL), and DMAP (14.7 mg, 0.120 mmol) and Et_3N (0.7 mL, 5 mmol) were added to the solution, followed by TBDMS-Cl (54.3 mg, 3.60 mmol). The resulting pale-yellow solution was stirred at rt for 48 h, and TLC analysis indicated conversion of the starting material to a less polar product. The reaction mixture was adsorbed onto silica gel and purified by flash chromatography in 1:9 EtOAc–hexanes to yield 870 mg (80%) of **20** as a clear oil (R_f 0.51, 2:8 EtOAc–hexanes). ^1H NMR (500 MHz, $CDCl_3$): δ 0.079 (s, 3H), 0.93 (s, 9H), 2.57 (br s, 1H), 3.60 (dd, 1H, $J = 9.2$, 3.4), 3.64–3.73 (m, 5H), 3.77 (dd, 1H, $J = 11.2$, 2.8), 3.96 (app. t, 1H, $J = 9.2$), 4.06 (app. dt, 1H, $J = 9.7$, 3.6), 4.15 (dd, 1H, $J = 9.7$, 4.8), 4.18 (app. t, 1H, $J = 9.2$), 4.28–4.35 (m, 1H), 4.77–4.91 (m, 6H), 5.01 (d, 1H, $J = 11.2$), 5.05 (d, 1H, $J = 11.2$), 5.23 (d, 2H, $J = 3.5$), 5.60 (s, 1H), 7.30–7.46 (m, 23H), 7.54–7.57 (m, 2H). ^{13}C DEPT135 NMR (125 MHz, $CDCl_3$): δ −5.2, −5.1, 26.2, 63.1, 63.3 (CH_2), 69.2 (CH_2), 71.2, 71.6, 73.2 (CH_2), 73.6 (CH_2), 75.5 (CH_2), 75.6 (CH_2), 78.8, 79.1, 79.4, 81.3, 82.6, 94.3, 94.9, 101.4, 126.4, 127.81, 127.86, 127.89, 127.95, 127.96, 127.97, 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 128.8, 129.1. HRFABMS (*m*/*z*): $[M+Li]^+$ calcd for $C_{53}H_{64}O_{11}SiLi$, 911.4378; found, 911.4354.

4.3.12. 2,3,2′,3′-Tetra-*O*-benzyl-4,6-benzylidene-4′-deoxy-6′-*O*-*tert*-butyldimethylsilyl-α,α-D-trehalose (21). Compound **20** (1.33 g, 1.47 mmol) was dissolved in dry MeCN (40 mL), and DMAP (0.717 g, 5.87 mmol) and PTC-Cl (0.40 mL, 2.9 mmol) were added to this solution. The reaction mixture was stirred at rt overnight, after which TLC analysis indicated the formation of a new, slightly less polar product as well as unreacted starting material. Complete consumption of the starting material could not be achieved even after adding more PTC-Cl and DMAP. At this point, the reaction mixture was concentrated and adsorbed onto silica gel and partially purified by flash chromatography (1:9 EtOAc–hexanes). This semi-purified product was dissolved in dry toluene (60 mL), and AIBN (50 mg, 0.30 mmol) and Bu_3SnH (1.9 mL, 6.0 mmol) were added. The resulting solution was heated at reflux for 15 min. After cooling to rt, the crude reaction mixture was adsorbed onto

silica gel and purified by flash chromatography (1:9 EtOAc–hexanes) to yield 743 mg (58%, two steps) of **21** as an amorphous white solid (R_f 0.56, 2:8 EtOAc–hexanes). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.034 (s, 3H), 0.041 (s, 3H), 0.89 (s, 9H), 1.51 (dd, 1H, $J = 13$, 12.2), 2.05–2.11 (m, 1H), 3.51–3.53 (m, 3H), 3.61–3.69 (m, 3H), 4.03–4.09 (m, 1H), 4.10–4.20 (m, 3H), 4.27–4.33 (m, 1H), 4.68–4.78 (m, 5H), 4.84 (d, 2H, $J = 11.1$), 4.95 (d, 1H, $J = 11.2$), 5.21 (d, 1H, $J = 2.4$), 5.22, (d, 1H, $J = 3.2$), 5.56 (s, 1H), 7.24–7.43 (m, 23H), 7.51 (dd, 2H, $J = 8.2$, 2.1). ^{13}C DEPT135 NMR (125 MHz, $CDCl_3$): δ −5.1, −3.3, 26.2, 33.5 (CH_2), 62.9, 65.9 (CH_2), 69.0, 69.3 (CH_2), 72.4, 73.56 (CH_2), 73.59 (CH_2), 75.5 (CH_2), 75.7, 78.8, 79.4, 80.5, 82.7, 94.2, 94.9, 101.5, 126.4, 127.8, 127.9, 128.0, 128.1, 128.2, 128.4, 128.5, 128.6, 128.7, 129.1. HRFABMS (m/z): $[M+Li]^+$ calcd for $C_{53}H_{64}O_{10}SiLi$, 895.4429; found, 895.4411.

4.3.13. 4-Deoxy-α,α-D-trehalose (8). Compound **21** (360 mg, 0.41 mmol) was dissolved in 20 mL of 9:1 MeOH–EtOAc with 10% AcOH (v/v). The solution was purged under N_2 for 10 min, and subjected to hydrogenolysis over $Pd(OH)_2$/C. (Caution! Extreme fire hazard.) After 3.5 h, most of the starting material had been converted to two new, more polar spots (R_f 0.76 and 0.28, 7:2:1 EtOAc–MeOH–H_2O). An additional 0.5 mL of AcOH was added to the reaction mixture, and the reaction vessel was heated at 50 °C overnight. The reaction appeared to be complete, and the reaction mixture was filtered through Celite to remove the Pd catalyst. The filtrate was concentrated and purified by flash chromatography (7:2:1 EtOAc–MeOH–H_2O) to yield a clear oil, from which residual water was removed by lyophilization to yield 55 mg (45%) of **8** as a fluffy, white solid. NMR data are summarized in Tables 1–3. HRFABMS (m/z): $[M+Na]^+$ calcd for $C_{12}H_{22}O_{10}Na$, 349.1111; found, 349.1119.

4.3.14. 2,2′-Di-*O*-benzoyl-4,6:4′,6′-di-*O*-benzylidene-α,α-D-trehalose (22). To a solution of 5.0 g (9.6 mmol) of **15** in 100 mL of dry MeCN was added 2.3 mL (19 mmol) of benzoyl cyanide followed by 0.5 mL (3 mmol) of Et_3N. After stirring for 2 h, the yellow reaction mixture was adsorbed onto silica gel and subjected to flash chromatography using 5:95 to 10:90 EtOAc–toluene to provide 3.15 g (45%) of the desired dibenzoate **22** (R_f 0.28, 15:85 EtOAc–toluene), essentially free of other contaminating isomers. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.54 (d, 2H, $J = 3.2$), 3.49 (app. t, 2H, $J = 10.0$), 3.56 (app. t, 2H, $J = 9.6$), 3.60 (dd, 2H, $J = 4.8$, 10.0), 3.86 (app. dt, 2H, $J = 4.8$, 10.0), 4.39 (app. t, 2H, $J = 9.6$), 5.11 (dd, 2H, $J = 4.0$, 9.6), 5.40 (s, 2H), 5.48 (d, 2H, $J = 4.0$), 7.13–7.64 (m, 16H), 8.16 (app. d, 4H, $J = 7.2$). ^{13}C DEPT135 NMR (125 MHz, $CDCl_3$) δ 63.2, 68.4 (CH_2), 69.0, 73.7, 81.2, 93.9, 102.1, 126.7, 128.4, 129.1, 129.6, 130.1, 133.9. Lit.:[57] ^{1}H NMR (400 MHz): δ 2.66 (br s, 2H), 3.49 (t, 2H, $J = 10.0$), 3.56 (t, 2H, $J = 9.6$), 3.60 (dd, 2H, $J = 4.8$, 10.0), 3.86 (dt, 2H, $J = 4.8$, 10.0), 4.39 (t, 2H, $J = 9.6$), 5.11 (dd, 2H, $J = 4.0$, 9.6), 5.40 (s, 2H), 5.48 (d, 2H, $J = 4.0$), 7.13–7.64 (m, 16H), 8.16 (d, 4H, $J = 7.2$).

4.3.15. 2,2′-Di-*O*-benzoyl-4,6:4′,6′-di-*O*-benzylidene-3,3′-dideoxy-α,α-D-trehalose (23). Compound **22** (0.300 g, 0.413 mmol) and DMAP (0.202 g, 1.65 mmol) were dissolved in dry MeCN (20 mL), and PTC-Cl (0.12 mL, 0.91 mmol) was added. The resulting bright-yellow solution was stirred at rt overnight, after which all starting material had been consumed as shown by TLC analysis; two major products had formed (R_f 0.46 and 0.58, 3:7 EtOAc–hexanes), presumably the mono- and diacylated species. The reaction mixture was adsorbed onto silica gel, after which purification by flash chromatography with 2:8 EtOAc–hexanes afforded 0.33 g (80%) of the R_f 0.58 spot as a white powder. This thionocarbonate (0.276 g, 0.277 mmol) was dissolved in 50 mL of dry toluene, AIBN (9 mg, 0.005 mmol) and Bu_3SnH (0.75 mL, 2.8 mmol) were added, and the solution was heated at reflux for 30 min. TLC analysis indicated complete conversion of the starting material to a new, less polar product. The reaction mixture was adsorbed onto silica gel, after which purification by flash chromatography (1:9 to 2:8 EtOAc–hexanes) yielded 0.161 g (84%) of **23** as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.38 (app. q, 2H, $J = 11.7$), 2.41–2.46 (m, 2H), 3.56 (app. t, 2H, $J = 10.4$), 3.70–3.77 (m, 2H), 3.75 (dd, 2H, $J = 10.6$, 4.8), 3.97 (app. td, 2H, $J = 10.0$, 4.8), 5.23–5.27 (m, 2H), 5.42 (d, 2H, $J = 3.4$), 5.47 (s, 2H), 7.34–7.43 (m, 14H), 7.54 (app. tt, 2H, $J = 7.5$, 1.3), 8.15 (app. dd, 4H, $J = 8.5$, 1.3). ^{13}C DEPT135 NMR (125 MHz, $CDCl_3$): δ 29.8 (CH_2), 65.1, 69.0 (CH_2), 69.2, 76.2, 92.7, 101.9, 126.6, 128.4, 129.0, 129.3, 130.0, 133.8. HRFABMS (m/z): $[M+Li]^+$ calcd for $C_{40}H_{38}O_{11}Li$, 701.2574; found, 701.2565.

4.3.16. 3,3′-Dideoxy-α,α-D-trehalose (5). Compound **23** (0.160 g, 0.230 mmol) was dissolved in dry MeOH (10 mL)–THF (2 mL), and a solution of 0.5 M NaOMe in MeOH (5 mL) was added. The reaction mixture was stirred at rt for 2 h, at which point TLC analysis indicated complete consumption of the starting material (R_f of product 0.31, 3:7 EtOAc–hexanes). The solvent was removed to yield a residue, which was redissolved in EtOAc and washed with H_2O and brine. The organic layer was concentrated and redissolved in 1:1 MeOH–TFA. After being stirred at rt for 10 min, complete consumption of the starting material was shown by TLC, and the reaction mixture was adsorbed onto silica gel. Purification by flash chromatography (7:2:1 EtOAc–MeOH–H_2O) yielded 13 mg (19%, two steps) of **5** as a white solid following lyophilization ($R_f = 0.56$, 7:2:1

EtOAc–MeOH–H_2O). NMR data are summarized in Tables 1–3. HRFABMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{12}H_{22}O_9Na$, 333.1162; found, 333.1170. Lit.:[37] ^1H NMR (300 MHz, MeOH-d_4): δ 5.08 (d, 1H, H-1), 3.8–3.5 (unresolved m, 5H, H-2, H-4, H-5 and two H-6), 2.09 (m, 1H, H-3b), 1.94 (m, 1H, H-3a, $J_{1,2} = 3.4$).

4.3.17. 2,2′-Dideoxy-3,3′-di-*O*-benzyl-4,6:4′,6′-di-*O*-benzylidene-α,α-D-trehalose (25). Compound **24** (0.650 g, 0.931 mmol) was dissolved in dry MeCN (30 mL), and DMAP (0.546 g, 4.47 mmol) and PTC-Cl (300 μL, 2.23 mmol) were added. The resulting bright-yellow solution was stirred at rt overnight, after which a white precipitate was observed and TLC analysis indicated complete conversion of the starting material to a new product. The precipitate was removed by filtration, and the filtrate was absorbed onto silica gel. Flash chromatography with 2:8 EtOAc–hexanes yielded 510 mg (56%) of a white foam. This product (0.40 g, 0.41 mmol) was dissolved in 60 mL of dry toluene, and AIBN (33 mg, 0.20 mmol) and Bu_3SnH (1.0 mL, 3.7 mmol) were added. The resulting clear solution was heated at reflux for 30 min, after which TLC analysis indicated complete consumption of the starting material. The reaction mixture was cooled to rt, absorbed onto silica gel and subjected to flash chromatography (2:8 EtOAc–hexanes) to yield 125 mg (91%) of **25** as a white amorphous solid (R_f 0.32, 2:8 EtOAc–hexanes), contaminated with a small amount of Sn-containing byproducts. ^1H NMR (500 MHz, $CDCl_3$): δ 1.84–1.90 (m, 2H), 2.23 (app. dd, 2H, $J = 14.0$, 4.9), 3.71–3.83 (m, 6H), 4.00–4.10 (m, 2H), 4.22–4.28 (m, 2H), 4.71 (d, 2H, $J = 11.9$), 4.86 (d, 2H, $J = 11.9$), 5.20 (d, 2H, $J = 3.5$), 5.65 (s, 2H), 7.26–7.43 (m, 16H), 7.52–7.55 (m, 4H). ^{13}C DEPT135 NMR (125 MHz, $CDCl_3$): δ 36.5 (CH_2), 64.3, 69.4 (CH_2), 73.1 (CH_2), 73.4 (CH_2), 84.1, 93.7, 101.8, 126.5, 128.09, 128.13, 128.7, 128.9, 129.4. HRFABMS (*m*/*z*): $[M+Li]^+$ calcd for $C_{40}H_{42}O_9Li$, 673.2989; found, 673.3002.

4.3.18. 2,2′-Dideoxy-α,α-D-trehalose (6). Compound **25** (215 mg, 0.320 mmol) was dissolved in 9:1 MeOH–AcOH (10 mL total volume) and subjected to hydrogenolysis using $Pd(OH)_2$/C. (Caution! Extreme fire hazard.) The reaction was monitored by TLC until all starting material was consumed. The Pd catalyst was removed by filtering through Celite, and the filtrate was adsorbed onto silica gel and purified by flash chromatography (7:2:1 EtOAc–MeOH–H_2O) to yield 91 mg (91%) of **6** as a white, fluffy solid following lyophilization. NMR data are summarized in Tables 1–3. HRFABMS (*m*/*z*): calcd for $C_{12}H_{22}O_9Na$, 333.1162; found, 333.1167.

4.3.19. 2-*O*-Benzoyl-4,6;2′,3′;4′,6′-tri-*O*-cyclohexylidene-α,α-D-trehalose (27). Compound **26** (0.612 g, 1.05 mmol), benzoic acid (0.128 g, 1.05 mmol), dicyclohexyl carbodiimide (DCC) (0.269 g, 1.26 mmol) and DMAP (0.141 g, 1.16 mmol) were dissolved in 25 mL of dry CH_2Cl_2. The solution was stirred at rt overnight, after which time a white precipitate was observed and TLC analysis indicated almost complete conversion of the starting material to a new product. The reaction mixture was concentrated, re-suspended in ice-cold acetone and filtered to remove insoluble urea byproduct. The filtrate was concentrated to yield an off-white residue, which was purified by flash chromatography (1:9 EtOAc–hexanes) to yield 0.58 g (80%) of **27** as a white solid (R_f 0.36, 2:8 EtOAc–hexanes). ^1H NMR (400 MHz, CD_3CN): δ 1.45–2.06 (m, 30H), 3.12 (dd, 1H, $J = 6.5$, 6.0), 3.40–3.43 (m, 1H), 3.51–3.58 (m, 3H), 3.71 (app. t, 1H, $J = 12.0$), 3.74–3.86 (m, 2H), 3.89 (app. t, 1H, $J = 11.5$), 3.90–3.97 (m, 1H), 4.03 (app. t, 1H, $J = 11.5$), 4.04–4.09 (m, 1H), 4.93 (dd, 1H, $J = 12.0$, 4.5), 5.35 (d, 1H, $J = 4.0$), 5.37 (d, 1H, $J = 4.5$), 7.52 (app. t, 2H, $J = 9.5$), 7.64 (app. t, 1H, $J = 9.5$), 8.12 (app. d, 2H, $J = 9.5$); ^{13}C DEPT135 NMR (125 MHz, CD_3CN): δ 23.2 (CH_2), 24.5 (CH_2), 26.0 (CH_2), 26.2 (CH_2), 28.5 (CH_2), 36.5 (CH_2), 36.9 (CH_2), 38.4 (CH_2), 38.8 (CH_2), 61.7 (CH_2), 62.1 (CH_2), 65.0, 67.1, 69.5, 73.2, 73.6, 74.4, 75.0, 76.7, 94.4, 94.8, 129.7, 130.5, 134.4. HRFABMS (*m*/*z*): $[M+Li]^+$ calcd for $C_{37}H_{50}O_{12}Li$, 693.3; found, 693.5. Anal. Calcd for $C_{37}H_{50}O_{12}$: C, 64.71; H, 7.34. Found: C, 64.44; H, 7.52.

4.3.20. 2-*O*-Benzoyl-4,6:2′,3′:4′,6′-tri-*O*-cyclohexylidene-3-deoxy-α,α-D-trehalose (28). Compound **27** (1.00 g, 1.46 mmol) and DMAP (0.356 g, 2.91 mmol) were dissolved in 50 mL of dry MeCN, and PTC-Cl (240 μL, 1.75 mmol) was added to this solution in one portion. The resulting bright-yellow solution was stirred at rt for 12 h, after which time a precipitate had formed. TLC analysis indicated complete consumption of the starting material. The reaction mixture was filtered through Celite, and the filtrate was concentrated and partially purified by flash chromatography (1:9 to 2:8 EtOAc–hexanes) to yield the thionocarbonate as a white foam (0.71 g, 59%). This foam (0.21 g, 0.26 mmol) was dissolved in 20 mL of dry toluene, and AIBN (8 mg, 0.05 mmol) and Bu_3SnH (0.34 mL, 1.3 mmol) were added. The resulting solution was heated at reflux for 30 min, at which time complete conversion of the thionocarbonate (R_f 0.41, 2:8 EtOAc–hexanes) to a new, slightly less polar spot (R_f 0.51, 2:8 EtOAc–hexanes) was observed. The reaction mixture was concentrated to yield a pale-yellow residue that was dissolved in 30 mL of MeCN and washed with pentane to remove the excess organotin byproducts. The crude product obtained was then purified by flash chromatography (1:9 to 2:8 EtOAc–hexanes) to yield 150 mg (88%) of **28** as a white solid. ^1H NMR (500 MHz, CD_3CN): δ 1.2–2.3 (m, 32H), 3.26 (dd, 1H, $J = 10.2$, 4.9), 3.45–3.91 (m, 1H), 3.54 (dd, 1H, $J = 9.4$, 3.0), 3.61 (app. t, 1H,

10.5), 3.72 (d, 2H, $J = 7.2$), 3.81–3.90 (m, 2H), 3.91 (app. t, 1H, $J = 9.3$), 4.05 (app. t, 1H, $J = 9.4$), 5.05–5.12 (m, 1H), 5.30 (d, 1H, $J = 3.4$), 5.41 (d, 1H, $J = 3.0$), 7.49 (app. t, 2H, $J = 8.0$), 7.61 (app. td, 1H, $J = 7.4$, 1.2), 8.04 (dd, 2H, $J = 8.0$, 1.2). ^{13}C DEPT135 NMR (125 MHz, CD_3CN): δ 22.3 (CH_2), 22.5 (CH_2), 22.6 (CH_2), 23.6 (CH_2), 25.1 (CH_2), 25.3 (CH_2), 27.6 (CH_2), 27.7 (CH_2), 29.8 (CH_2), 35.6 (CH_2), 36.0 (CH_2), 37.5 (CH_2), 37.8 (CH_2), 60.8 (CH_2), 61.5 (CH_2), 65.8, 66.1, 67.1, 69.2, 72.4, 73.5, 75.9, 92.2, 93.6, 128.7, 129.4, 133.4. HRFABMS (*m*/*z*): $[M+Li]^+$ calcd for $C_{37}H_{50}O_{11}Li$, 677.3513; found, 677.3527.

4.3.21. 3-Deoxy-α,α-D-trehalose (9). Compound **28** (120 mg, 0.179 mmol) was dissolved in 5 mL of anhyd MeOH, and 3.6 mL of a 0.5 M NaOMe solution in MeOH was added. The reaction mixture was stirred at rt for 5 h, after which TLC analysis indicated almost complete conversion of the starting material to the debenzoylated product. The reaction mixture was concentrated and redissolved in 20 mL of 1:1 10% aq HCl–THF and stirred at rt for 48 h. The residual acid was neutralized by the addition of Dowex 1 × 8 200–400 mesh resin (Cl^- form, exchanged to OH^- form). The resin was removed by filtration, and the filtrate was concentrated and purified by flash chromatography (7:2:1 EtOAc–MeOH–H_2O) to yield 35 mg (60%) of **9** as a white solid following lyophilization. NMR data are summarized in Tables 1–3. HRFABMS (*m*/*z*): $[M+Na]^+$ calcd for $C_{12}H_{22}O_{10}Na$, 349.1111; found, 349.1109.

4.3.22. 2-*O*-Benzoyl-4,6:2′,3′:4′,6′-tri-*O*-cyclohexylidene-3-*O*-methoxymethyl-α,α-D-trehalose (29). Compound **27** (0.617 g, 0.898 mmol) and tetrabutylammonium iodide (TBAI) (0.66 g, 1.8 mmol) were dissolved in 6 mL of freshly distilled Hünig's base (ethyldiisopropylamine). MOM-Cl (1.0 mL, 13 mmol) was added to the above solution. The reaction mixture was heated in a 55 °C oil bath for 2 h, after which an orange sticky tar was observed and TLC analysis indicated almost complete conversion of the starting material to a less polar compound (R_f 0.48, 2:8 EtOAc–hexanes). The reaction mixture was concentrated under high vacuum, and the resulting orange residue was redissolved in EtOAc. The organic layer was washed with water and brine and adsorbed onto silica gel. Purification by flash chromatography (1:9 to 2:8 EtOAc–hexanes) yielded 0.59 g (90%) of **29** as a white solid. ^{1}H NMR (500 MHz, CD_3CN): δ 1.28–1.66 (m, 27H), 1.85–1.95 (m, 1H), 1.99–2.07 (m, 1H), 2.17–2.25 (m, 1H), 3.09–3.14 (dd, 1H, $J = 10.5$, 5.5), 3.26 (s, 3H), 3.49 (dd, 1H, $J = 9.5$, 3.0), 3.50–3.53 (m, 2H), 3.74 (app. t, 1H, $J = 9.5$), 3.76 (app. t, 1H, $J = 9.5$), 3.82–3.89 (m, 2H), 4.10–4.18 (m, 3H), 4.71 (d, 1H, $J = 6.5$), 4.94 (d, 1H, $J = 6.6$), 5.13 (dd, 1H, $J = 9.5$, 4.0), 5.34 (d, 1H, $J = 3.0$), 5.39 (d, 1H, $J = 4.0$), 7.48 (t, 2H, $J = 8.0$), 7.59 (t, 1H, $J = 7.5$), 8.13 (d, 2H, $J = 8.0$); ^{13}C DEPT135 NMR (125 MHz, $CDCl_3$): δ 22.36 (CH_2), 22.44 (CH_2), 22.6 (CH_2), 23.5 (CH_2), 23.8 (CH_2), 25.5 (CH_2), 36.0 (CH_2), 36.2 (CH_2), 37.9 (CH_2), 55.6, 60.9 (CH_2), 61.6 (CH_2), 63.8, 66.2, 72.5, 72.7, 73.2, 73.4, 76.0, 76.7, 94.5, 94.9, 96.9 (CH_2), 128.7, 129.6, 133.4. LRFABMS (*m*/*z*): $[M+Li]^+$ calcd for $C_{39}H_{54}O_{13}Li$, 737; found, 737. Anal. Calcd for $C_{39}H_{54}O_{13}$: C, 64.09; H, 7.45. Found: C, 64.30; H, 7.62.

4.3.23. 4,6:2′,3′:4′,6′-Tri-*O*-cyclohexylidene-3-*O*-methoxymethyl-2-*O*-phenoxythionocarbonyl-α,α-D-trehalose (30). Compound **29** (0.175 g, 0.240 mmol) was dissolved in 5 mL of dry MeOH, and 0.5 mL of a solution of 0.5 M NaOMe in MeOH was added. Once the debenzoylation reaction was complete as shown by TLC, the reaction mixture was concentrated in vacuo. The resulting residue was resuspended in EtOAc and washed with NH_4Cl solution and brine. Upon removal of the solvent, the residue was redissolved in 20 mL of dry MeCN, and DMAP (0.058 g, 0.48 mmol) and PTC-Cl (35 μL, 0.26 mmol) were added. The resulting bright-yellow solution was stirred at rt overnight, after which TLC analysis indicated complete consumption of the starting material. The reaction mixture was concentrated and purified by flash chromatography (2:8 EtOAc–hexanes) to yield 0.137 g (75%) of **30** as a white solid (R_f 0.55, 2:8 EtOAc–hexanes). ^{1}H NMR (500 MHz, CD_3CN): δ 1.28–1.80 (m, 28H), 2.03–2.19 (m, 2H), 3.48 (s, 3H), 3.53 (dd, 1H, $J = 9.5$, 3.0), 3.55–3.90 (m, 6H), 3.95–4.01 (m, 2H), 4.14 (app. t, 1H, $J = 9.5$), 4.20 (app. t, 1H, $J = 9.5$), 4.76 (d, 1H, $J = 6.5$), 4.96 (d, 1H, $J = 6.5$), 5.25 (dd, 1H, $J = 9.5$, 4.0), 5.39 (d, 1H, $J = 3.0$), 5.69 (d, 1H, $J = 4.0$), 7.22 (d, 2H, $J = 7.5$), 7.27 (t, 1H, $J = 7.5$), 7.40 (t, 2H, $J = 7.5$); ^{13}C DEPT135 NMR (125 MHz, $CDCl_3$): δ 15.2 (CH_2), 22.4 (CH_2), 22.5 (CH_2), 22.7 (CH_2), 22.8 (CH_2), 23.5 (CH_2), 23.8 (CH_2), 24.98 (CH_2), 25.52 (CH_2), 27.7 (CH_2), 27.8 (CH_2), 35.9 (CH_2), 36.2 (CH_2), 37.7 (CH_2), 37.8 (CH_2), 55.7, 61.4 (CH_2), 61.5 (CH_2), 63.8, 66.3, 72.9, 73.0, 73.2, 76.0, 80.7, 91.8, 93.6, 97.0 (CH_2), 121.9, 126.6, 129.5. HRFABMS (*m*/*z*): $[M+Li]^+$ calcd for $C_{39}H_{54}O_{13}SLi$: 769.3445; found, 769.3444.

4.3.24. 2-Deoxy-α,α-D-trehalose (10). Compound **30** (0.23 g, 0.30 mmol) was dissolved in 20 mL of dry toluene. AIBN (10 mg, 0.006 mmol) and Bu_3SnH (0.40 mL, 1.5 mmol) were added to this solution, and it was heated at reflux for 30 min. At this point, a new product (R_f 0.40, 2:8 EtOAc–hexanes) was evident by TLC. The reaction mixture was concentrated in vacuo to yield a clear oil. This oil was redissolved in MeCN and washed with pentane to remove organotin byproducts. The MeCN layer was concentrated and dissolved in 1:1 10% aq HCl–THF. After stirring at rt overnight, deprotection appeared to be complete by TLC. The residual

acid was neutralized by addition of Dowex 1 × 8 200–400 mesh resin (Cl^- form, exchanged to OH^- form). The resin was removed by filtration. The filtrate was concentrated and purified by flash chromatography (7:2:1 EtOAc–MeOH–H_2O) to yield 40 mg (40%, two steps) of **10** as a white solid following lyophilization (R_f 0.32, 7:2:1 EtOAc–MeOH–H_2O). NMR data are summarized in Tables 1–3. HRFABMS (*m*/*z*): $[M+H]^+$ calcd calcd for $C_{12}H_{23}O_{10}$, 327.1291; found, 327.1289.

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Supplementary data

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References

1. Wolf, A.; Krämer, R.; Morbach, S. *Mol. Microbiol.* **2003**, *49*, 1119–1134.
2. Singer, M. A.; Lindquist, S. *TIBTECH* **1998**, *16*, 460–468.
3. Benaroudj, N.; Lee, D. H.; Goldberg, A. L. *J. Biol. Chem.* **2001**, *276*, 24261–24267.
4. Elbein, A. D.; Pan, Y. T.; Pastuszak, I.; Carroll, D. *Glycobiology* **2003**, *13*, 17R–27R.
5. De Smet, K. A. L.; Weston, A.; Brown, I. N.; Young, D. B.; Robertson, B. D. *Microbiology* **2000**, *146*, 199–208.
6. Elbein, A. D.; Mitchell, M. *J. Bacteriol.* **1973**, *113*, 863–873.
7. Besra, G. S.; Chatterjee, D. Lipids and Carbohydrates of *Mycobacterium tuberculosis*. In *Tuberculosis: Pathogenesis, Protection and Control*; Bloom, B. R., Ed.; American Society for Microbiology: Washington, DC, 1994; pp 285–306.
8. Dubos, R. J.; Middlebrook, G. *Am. Rev. Tuberc.* **1948**, *58*, 698–699.
9. Middlebrook, G.; Coleman, C. M.; Schaefer, W. B. *Proc. Natl. Acad. Sci. U.S.A.* **1959**, *45*, 1801–1804.
10. Goren, M. B.; Brokl, O.; Roller, P.; Fales, H. M.; Das, B. C. *Biochemistry* **1976**, *15*, 2728–2735.
11. Goren, M. B.; Brokl, O.; Das, B. C.; Lederer, E. *Biochemistry* **1971**, *10*, 72–81.
12. Goren, M. B. *Biochim. Biophys. Acta* **1970**, *210*, 127–138.
13. Goren, M. B. *Biochim. Biophys. Acta* **1970**, *210*, 116–126.
14. Goren, M. B.; D'Arcy Hart, P.; Young, M. R.; Armstrong, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 2510–2514.
15. Zhang, L.; English, D.; Andersen, B. R. *J. Immunol.* **1991**, *146*, 2730–2736.
16. Pabst, M. J.; Gross, J. M.; Brozna, J. P.; Goren, M. B. *J. Immunol.* **1988**, *140*, 634–640.
17. Gilleron, M.; Stenger, S.; Mazorra, Z.; Wittke, F.; Mariotti, S.; Bohmer, G.; Prandi, J.; Mori, L.; Puzo, G.; De Libero, G. *J. Exp. Med.* **2004**, *199*, 649–659.
18. Mougous, J. D.; Petzold, C. J.; Senaratne, R. H.; Lee, D. H.; Akey, D. L.; Lin, F. L.; Munchel, S. E.; Pratt, M. R.; Riley, L. W.; Leary, J. A.; Berger, J. M.; Bertozzi, C. R. *Nat. Struct. Mol. Biol.* **2004**, *11*, 721–729.
19. Hadfield, A. F.; Hough, L.; Richardson, A. C. *Carbohydr. Res.* **1978**, *63*, 51–60.
20. Hadfield, A. F.; Hough, L.; Richardson, A. C. *Carbohydr. Res.* **1980**, *80*, 123–130.
21. Rose, J. D.; Maddry, J. A.; Comber, R. N.; Suling, W. J.; Wilson, L. N.; Reynolds, R. C. *Carbohydr. Res.* **2002**, *337*, 105–120.
22. Azuma, I.; Sakurai, T.; Ishida, H.; Kitajima, T.; Yamazaki, M. *Carbohydr. Res.* **1991**, *212*, 47–53.
23. Wang, J.; Elchert, B.; Hui, Y.; Takemoto, J. Y.; Bensaci, M.; Wennergren, J.; Chang, H.; Rai, R.; Chang, C.-W. T. *Bioorg. Med. Chem.* **2004**, *12*, 6397–6413.
24. Hui, Y.; Chang, C.-W. T. *Org. Lett.* **2002**, *4*, 2245–2248.
25. Birch, G. G. *J. Chem. Soc. C* **1966**, 1072–1074.
26. Birch, G.; Richardson, A. C. *Carbohydr. Res.* **1968**, *8*, 411–415.
27. Birch, G. G.; Lee, C.-K.; Richardson, A. C. *Carbohydr. Res.* **1974**, *36*, 97–109.
28. Hanessian, S.; Lavallée, P. *Carbohydr. Res.* **1973**, *28*, 303–311.
29. Hough, L.; Munroe, P. A.; Richardson, A. C. *J. Chem. Soc. C* **1971**, 1090–1094.
30. Hough, L.; Richardson, A. C.; Tarelli, E. *J. Chem. Soc. C* **1971**, 1732–1738.
31. Hough, L.; Richardson, A. C.; Tarelli, E. *J. Chem. Soc. C* **1971**, 2122–2127.
32. Pratt, M. R.; Leigh, C. D.; Bertozzi, C. R. *Org. Lett.* **2003**, *5*, 3185–3188.
33. Robins, M. J.; Wilson, J. S. *J. Am. Chem. Soc.* **1981**, *103*, 932–933.
34. Robins, M. J.; Wilson, J. S.; Hansske, F. *J. Am. Chem. Soc.* **1983**, *105*, 4059–4065.
35. Hanessian, S.; Plessas, N. R. *J. Org. Chem.* **1969**, *34*, 1035–1044.
36. Garegg, P. J.; Hultberg, H.; Wallin, S. *Carbohydr. Res* **1982**, *108*, 97–101.
37. Lichtenthaler, F. W.; Werner, B. *Carbohydr. Res.* **1999**, *319*, 47–54.
38. Birch, G. G.; Lee, C. K.; Richardson, A. C.; Ali, Y. *Carbohydr. Res.* **1976**, *49*, 153–161.
39. Holy, A.; Soucek, M. *Tetrahedron Lett.* **1971**, 185–188.
40. Wang, C.-C.; Lee, J.-C.; Luo, S.-Y.; Fan, H.-F.; Pai, C.-L.; Yang, W.-C.; Lu, L.-D.; Hung, S.-C. *Angew. Chem., Int. Ed.* **2002**, *41*, 2360–2362.
41. Wallace, P. A.; Minnikin, D. E. *Carbohydr. Res.* **1994**, *263*, 43–59.
42. Wallace, P. A.; Minnikin, D. E. *J. Chem. Soc., Chem. Commun.* **1993**, 1292–1293.
43. Stork, G.; Takahashi, T. *J. Am. Chem. Soc.* **1977**, *99*, 1275–1276.
44. Van Halbeek, H. In *Encyclopedia of Nuclear Magnetic Resonance*; Grant, D. M., Harris, R. K., Eds.; Wiley: New York, 1996; pp 1107–1137.

45. Duus, J. Ø.; Gotfredsen, C. H.; Bock, K. *Chem. Rev.* **2000**, *100*, 4589–4614.
46. Piantini, U.; Sørensen, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 6800–6801.
47. Hurd, R. E. *J. Magn. Reson.* **1990**, *87*, 422–428.
48. Von Kienlin, M.; Moonen, C. T. W.; Van der Toorn, A.; Van Zijl, P. C. M. *J. Magn. Reson.* **1991**, *93*, 423–429.
49. Braunschweiler, R.; Ernst, R. R. *J. Magn. Reson.* **1983**, *53*, 521–528.
50. Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355–360.
51. Marion, D.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 967–974.
52. Bodenhausen, G.; Ruben, D. J. *Chem. Phys. Lett.* **1980**, *69*, 185–189.
53. Palmer, A. G., III; Cavanagh, J.; Wright, P. E.; Rance, M. *J. Magn. Reson.* **1991**, *93*, 151–170.
54. Kay, L. E.; Keifer, P.; Saarinen, T. *J. Am. Chem. Soc.* **1992**, *114*, 10663–10665.
55. Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093–2094.
56. Cicero, D. O.; Barbato, G.; Bazzo, R. *J. Magn. Reson.* **2001**, *148*, 209–213.
57. Baer, H. H.; Bell, A. J. *Carbohydr. Res.* **1979**, *75*, 175–184.