ELSEVIER

Carbohydrate Research 263 (1994) 43–59

CARBOHYDRATE RESEARCH

Synthesis of 4,6:2′,3′:4′,6′-tri-*O*-cyclohexylidene-α,α'-trehalose 2-palmitate: an intermediate for the synthesis of mycobacterial 2,3-di-*O*-acyl-α,α'-trehalose antigens

Paul A. Wallace, David E. Minnikin *

Department of Chemistry, University of Newcastle, Newcastle upon Tyne, NE1 7RU, United Kingdom

Received 16 August 1993; accepted in revised form 1 April 1994

Abstract

The aim was to 'triprotect' trehalose by placing various acetals, or related protecting groups, across the 4,6-, 2′,3′-, and 4′,6′-positions, leaving the 2,3-positions free for subsequent acylation. Isopropylidene and ethylidene acetals were studied, with the formation of a small amount of 4,6:2′,3′:4′,6′-tri-*O*-isopropylidene-α,α'-trehalose. 4,6:4′,6′-Di-*O*-benzylidene-2′,3′-*O*-(tetraphenyldisiloxane-1,3-diyl)-α,α'-trehalose 2,3-diacetate was prepared in low yield. 1,1-Dimethoxycyclohexane reacted with methyl α-D-glucopyranoside to afford the 4,6-*O*-cyclohexylidene derivative, isolated as the diacetate; mild acid cleavage of the acetal gave the 2,3-diacetate. 4,6:2′,3′:4′,6′-Tri-*O*-cyclohexylidene-α,α'-trehalose is the major product of the reaction between α,α'-trehalose and 1,1-dimethoxycyclohexane. 2,3:4,6:2′,3′:4′,6′-Tetra-*O*-cyclohexylidene-, 4,6:4′,6′-di-*O*-cyclohexylidene-, and 4,6-*O*-cyclohexylidene-α,α'-trehaloses were also isolated in lower yields, all acetals being characterised as their peracetates. The proportions of the different trehalose acetals were dependent upon the molar ratio of 1,1-dimethoxycyclohexane and particularly on the reaction temperature. The triprotected trehalose acetal was acylated with palmitic acid, with excellent regioselectivity, affording the 2-*O*-palmitoyl ester. This 2-monoacylated, triprotected trehalose is a key intermediate for the synthesis of 2,3-di-*O*-acyl-α,α'-trehalose glycolipid antigens, isolated from *Mycobacterium fortuitum* and *Mycobacterium tuberculosis*.

Keywords: 2,3-Di-*O*-acyl-α,α'-trehalose; Glycolipid antigens; Cyclohexylidene acetals; 4,6:2′,3′:4′,6′-Tri-*O*-cyclohexylidene-α,α'-trehalose; C-2 regioselective esterification

1. Introduction

There has been an alarming increase in reported cases of tuberculosis, due primarily to the onset of acquired immune deficiency syndrome (AIDS). Immunosuppression in patients

* Corresponding author.

SSDI 0008-6215(94)00164-B

encourages the development of drug resistant strains of the tubercle bacillus, *Mycobacterium tuberculosis* [1]. The major problem with such mycobacterial diseases is the long time required to detect the particular infection. Many newly-emerging detection techniques require synthetic antigens as standards. It has therefore become necessary to develop synthetic methods that can provide large quantities of antigenic glycolipids which have been isolated from the cell envelope, to improve the detection methods available for the disease.

Recently, Minnikin et al. [2], Besra et al. [3], Lemassu et al. [4], and Baer [5] identified a series of 2,3-di-*O*-acyl-α,α'-trehaloses from *M. tuberculosis*. Also, studies on *Mycobacterium fortuitum* by Gautier et al. [6], Hamid et al. [7], and Sempere et al. [8] have revealed the presence of other acyltrehaloses, including 2,3-di-*O*-acyltrehaloses. All of these lipids have been identified as being strongly antigenic. The trehalose-based glycolipid antigens from *M. tuberculosis* have the 2-position esterified by a straight-chain (C_{16} or C_{18}) fatty acid and the 3-position of the same glucosyl unit esterified by a more complex long-chain fatty acid [3].

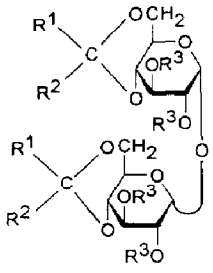
Recently, Baer and Wu [9] have developed a synthetic scheme for 2,3-di-*O*-acyl-α,α'-trehaloses involving selective acylation of the 2,3-*O*-dibutylstannylene derivative of 4,6:4′,6′-di-*O*-benzylidene-α,α'-trehalose, protection of the 2′,3′-diol system of the monoester by cyclising silylation with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, further acylation, and deprotection.

In this study we report the development of a simple trehalose 'triprotection' strategy which leaves the 2- and 3-positions of one glucosyl unit available for regioselective acylation. Parts of this work have been the subject of a preliminary publication [10].

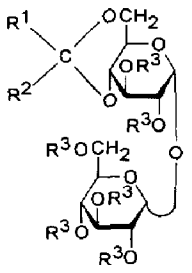
2. Results and discussion

It was initially planned to attempt a so-called 'triprotection' of α,α'-trehalose, such that the 2- and the 3-positions of the same glucosyl residue remained vacant, while the 2′,3′-, 4,6-, and 4′,6′-positions were protected as three cyclic acetals. This process appeared to be a more direct approach than attempting glycoside coupling reactions to form the disaccharide or using two chemically different forms of protecting groups. Preliminary results, using monosaccharide models, demonstrated that fatty acids could be esterified to the free hydroxyl groups of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**23**) with very satisfactory results [11]. The products gained were easily separable by column chromatography and the regioisomers relatively straightforward to identify from their ^{1}H NMR spectra.

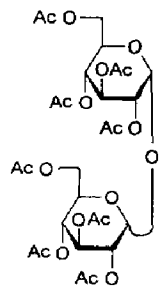
Initially, anhydrous α,α'-trehalose was required for most of the acetal protecting schemes. This was obtained via a modified procedure of Birch [12] by removal of the two moles of water of crystallisation using pyridine distillation. Reaction of anhydrous α,α'-trehalose with 4.1 molar equivalents of 2-methoxypropene in anhydrous DMF resulted in the formation of three well-characterised products, isolated as their acetate esters. These were the 'triprotected', 'diprotected', and 'monoprotected' isopropylidene acetals of α,α'-trehalose, i.e., compounds **12**, **1**, and **9**, respectively. Compounds **1** and **9** were described in previous studies [13], but compound **12** is reported for the first time. The mass spectra of these molecules were particularly useful due to the cleavage of the disaccharide across the central



	R^1	R^2	R^3
1	Me	Me	Ac
2	Me	Me	H
3	H	Me	Ac
4	H	Ph	H
5	H	Ph	Ac
6	-$(CH_2)_5$-		Ac
7	-$(CH_2)_5$-		H



	R^1	R^2	R^3
8	H	Me	Ac
9	Me	Me	Ac
11	-$(CH_2)_5$-		Ac



10

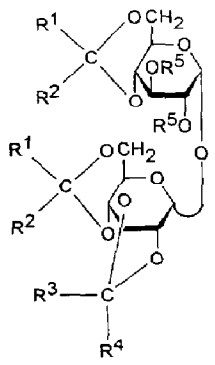
oxygen atom, to give two ions. Mass spectra of most of the disaccharides in this paper showed similar behaviour.

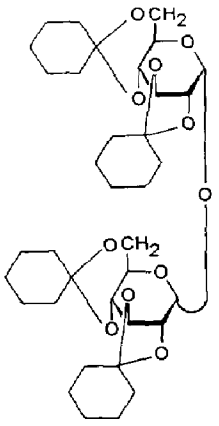
The most useful information to determine the substitution pattern about the trehalose skeleton was gained from the shift of the anomeric protons (H-1 and H-1′) and anomeric carbons (C-1 and C-1′), in both the ^1H and ^{13}C NMR spectra. If an unsymmetrically substituted trehalose was formed (i.e., triprotected or monoprotected), then a characteristic pair of doublets was observed, centered on δ 5.0–5.3. The couplings of the anomeric protons were usually in the region of J 3.1–4.7 Hz. Also, the shifts and coupling patterns of the H-2/2′ and H-3/3′ ring protons were very useful, depending on the substituted trehalose formed. This was especially so in the determination of the position of the long-chain fatty acid after the monoesterification reaction (**19**).

A small quantity of the desired 'triprotected' product (**12**) was formed from the reaction described, but the major product was the 4,6:4′,6′-di-*O*-isopropylidene-α,α'-trehalose **1**. Unfortunately, the yields of this reaction appeared to be variable and a yield of **12** suitable for the synthetic process required proved difficult to achieve, even when using an excess of 2-methoxypropene and several changes of reaction conditions.

The *O*-acetyl groups could easily be removed, using NaOMe in MeOH--$CHCl_3$. This yielded the free hydroxyl groups of the tri- and di-protected α,α'-trehaloses **2** and **13**, respectively. Attempts were then made to form the triprotected isopropylidene acetal by using 2,2-dimethoxypropane and acetone with various catalysts and under conditions ranging from 0°C to a vigorous reflux. None of the desired product was formed, only 4,6:4′,6′-di-*O*-isopropylidene-α,α'-trehalose (**2**).

For the isopropylidene derivatives **1**, **2**, **9**, **12**, and **13**, the methyl groups of the isopropylidene acetals were observed in the expected range of δ 1.4–1.5. Acetate methyl groups were observed at δ 1.9–2.2 in all cases, with a series of descending shifts from the 2-*O*-acyl to the 6-*O*-acyl (δ 2.2–1.9) for the trehalose octa-acetate (**10**).





16

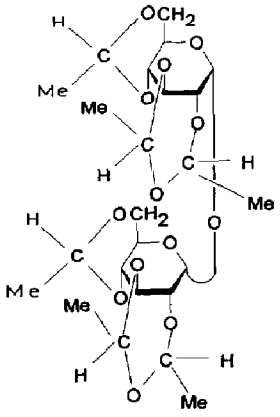
	R^1	R^2	R^3	R^4	R^5
12	Me	Me	Me	Me	Ac
13	Me	Me	Me	Me	H
14	-$(CH_2)_5$-		-$(CH_2)_5$-		Ac
15	-$(CH_2)_5$-		-$(CH_2)_5$-		H

Our attention then turned to the use of a more reactive species, which may form acetals across the 2,3-*trans*-diol systems found in trehalose. Therefore, acetaldehyde and paraldehyde were used in an attempt to produce ethylidene acetals. This process of acetalation was carried out in either paraldehyde or acetaldehyde with concentrated sulphuric acid catalysis and using different quantities of reagent and varied reaction conditions [14]. The isolated products, again as acetates, were diprotected (**3**), monoprotected (**8**), and an interesting tetraprotected trehalose (**17**) with no product corresponding to the desired triprotected compound.

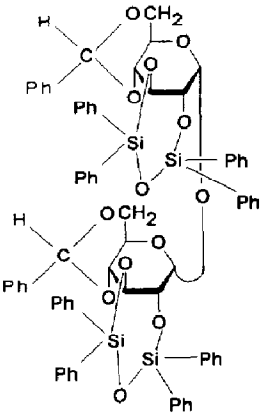
The tetraprotected ethylidene trehalose species (**17**) gave a very characteristic complex spectrum, with four unresolved multiplets composing the main part of the spectrum. The tetraprotected ethylidene derivative (**17**) is very characteristic of α,α'-trehalose since it is the only naturally occurring disaccharide which is capable of forming such a derivative [14]. This proved to be a good standard for the purposes of GLC and TLC for the identification of trehalose in degraded natural products isolated from mycobacteria, being possibly superior to trehalose octa-acetate.

Attention was turned to the use of a 4,6:4′,6′-α,α'-trehalose diacetal, with the possibility of adding a third acetal across one of the 2,3-*trans*-diol units. 4,6:4′,6′-Di-*O*-benzylidene-α,α'-trehalose (**4**) was prepared as the starting material using the standard conditions of zinc chloride and benzaldehyde. This substance was characterised as its tetraacetate (**5**) and the bulk (**4**) was recrystallised from a solution of 1% ammonia in water [15].

In preliminary experiments (results not shown), we unsuccessfully attempted to place standard acetal-protecting groups, such as isopropylidene or ethylidene, on to one of the two vacant 2,3-*trans*-diol positions of 4,6:4′,6′-di-*O*-benzylidene-α,α'-trehalose (**4**). In most cases, either starting material was recovered or the two benzylidene groups were removed by the acid catalyst. Attention was then turned to the formation of acetals which required base catalysis, such as the use of silicon-containing acetals, with methyl 4,6-*O*-



17



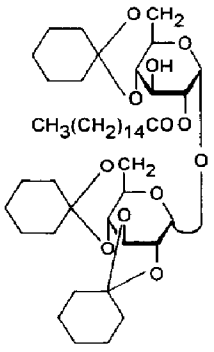
18

benzylidene-α-D-glucopyranoside (**23**) [16] as a model compound. The two silyl reagents of interest were dichlorodiphenylsilane and di-*tert*-butyldichlorosilane [17,18]. The products, obtained in reasonable yield, were similar to those obtained when 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPS) [9,19–21] was used to form acetals across 1,2-*trans*-diols. The inclusion of the oxygen in the silicon-containing acetal derivatives may be due to the reaction with water during the aqueous work-up.

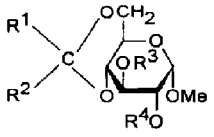
We then attempted to place these two silicon reagents across both of the diol units in the benzylidene-protected trehalose. This used an excess of the reagent and the best conditions were found to be 20°C. When two equivalents of the protecting group were used, in an attempt to form such a system across only one of the two diol units, two products were obtained. These were the 4,6:4′,6′-di-*O*-benzylidene-2,3:2′,3′-di-*O*-(tetraphenyldisiloxane-1,3-diyl)-α,α'-trehalose (**18**) and a small quantity of the desired 4,6:4′,6′-di-*O*-benzylidene-2′,3′-*O*-(tetraphenyldisiloxane-1,3-diyl)-α,α'-trehalose, isolated as its diacetate (**25**). No reaction occurred with di-*tert*-butyldichlorosilane. Changes of conditions, such as the use of high dilution and slow addition techniques, and also changes in the reaction time and temperature did not affect the low yields of the desired product. This protection strategy was therefore abandoned.

For the benzylidene-protected trehaloses **4**, **5**, **18**, and **25**, the anomeric protons were shifted slightly to ca. δ 5.32, whereas in the monosaccharides **23** and **24** the shift of the same proton was ca. δ 4.93. The acetal proton, PhC*H*, was at δ 5.41 in the monosaccharides and at δ 5.53 in the symmetrical disaccharide. Interestingly, with the unsymmetrical silylated benzylidene-trehalose **25**, a clear separation of this acetal proton was observed, the proton for the silylated glucosyl portion appearing at a lower shift than for the di-*O*-acetylglucosyl portion (δ 5.47 compared to 5.36).

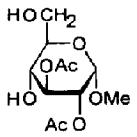
Work by Bissett et al. [22] had shown that it was possible to use cyclohexylidene acetals to protect both the 4,6- and 2,3-diols in glucose and other monosaccharides. We prepared the active reagent 1,1-dimethoxycyclohexane, using methoxytrimethylsilane and catalytic trimethylsilyl trifluoromethanesulfonate according to the procedure of Tsumoda et al. [23], in 90% overall yield. Methyl α-D-glucopyranoside was then used as a model compound for α,α'-trehalose. Reaction at room temperature afforded the 4,6-*O*-cyclohexylidene deriva-



19



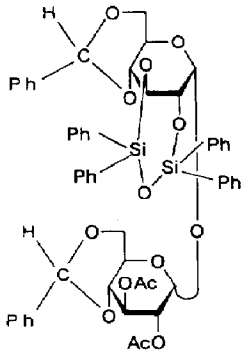
	R1	R2	R3	R4
20	-(CH2)5-		Ac	Ac
21	-(CH2)5		H	H
23	H	Ph	H	H
24	H	Ph	Ac	Ac



22

tive, isolated as its diacetate (**20**). Reaction at elevated temperatures had been shown to afford the 2,3:4,6-di-*O*-cyclohexylidene derivative [22]. Cleavage of the protecting acetal, using a 1:1 mixture of THF–aqueous 10% hydrochloric acid, had no effect on the two acetate esters. This indicated that if a tri-*O*-cyclohexylidene species could be formed and acylated, then the protecting groups could be easily removed with no effect upon the acyl groups.

Anhydrous α,α'-trehalose was dissolved in DMF and heated to reflux under reduced pressure using 3 molar equivalents of 1,1-dimethoxycyclohexane and a catalytic quantity of *p*-toluenesulfonic acid. Isolation of the mixture, followed by acetylation, produced only the di- and mono-protected trehalose acetates. Hence, we then attempted the same reaction, but using 8 molar equivalents of 1,1-dimethoxycyclohexane. The crude product mixture was again acetylated; the resulting mixture was then easily separable using column chromatography, the major product being the desired 2,3-di-*O*-acetyl-4,6:2′,3′:4′,6′-tri-*O*-cyclohexylidene-α,α'-trehalose (**14**).



25

In the case of the triprotected cyclohexylidene compound **14**, a double doublet, centred on δ 4.78, with $J_{1,2}$ 4.0 Hz and $J_{2,3}$ 9.1 Hz is characteristic of H-2 and a triplet centred on δ 5.41 with $J_{2,3} = J_{3,4} = 9.1$ Hz (for H-3) occurs in a 2,3-di-*O*-acylated trehalose. In the case of a 2-*O*-acylated, triprotected trehalose (**19**), the triplet for H-3 is not observed in the expected region, indicating a regioselective 2-*O*-acylation. In addition, in the ^{13}C NMR spectrum of **19** the shift of C-3 does not correspond to that for an esterified carbon atom. For the anomeric carbon atoms in compounds **6**, **7**, and **16**, the ^{13}C NMR spectra had one signal (δ ca. 90) indicating a symmetrically substituted trehalose or two signals, in compounds **11**, **14**, and **15** (δ ca. 90–92), indicating an unsymmetrically substituted trehalose.

This suitably protected trehalose **14** was then subjected to acetate cleavage, using the conditions mentioned above. The resulting product (**15**) was stable to column chromatography and was fully characterised. Esterification of this substrate using palmitic acid, dicyclohexylcarbodiimide, and 4-dimethylaminopyridine [24] in CH_2Cl_2 gave only one observable product on TLC (**19**). Three minor products were just visible, but there was insufficient material to allow characterisation. NMR and COSY–NMR experiments confirmed that the fatty acid had been selectively esterified to the 2-position.

The signals due to cyclohexylidene groups in compounds **6**, **7**, **11**, **14**, **15**, **16**, **19**, **20**, and **21** remained fairly unresolved in the 200-MHz ^1H NMR spectra, but COSY spectra at 500 MHz gave evidence of two well-resolved multiplets at δ ca. 1.40 and 1.60. These were observed as characteristic broad bands in the 200-MHz spectra. The carbon spectra of these cyclohexylidene derivatives provided some interesting results. Each carbon atom in the cyclohexylidene ring appeared at a slightly different shift (due to its unsymmetrical nature when attached to the carbohydrate), whereas 1,1-dimethoxycyclohexane itself showed four signals for the six ring carbons in four different chemical environments. A clear difference in the shifts of the carbon atoms within these rings was also observed, depending on whether the acetal was protecting a 2,3- or 4,6-diol (due to different environments of the acetal-forming carbon) [28]. Also observed was a distinct 'doublet'-type signal for the C-4 carbon of the cyclohexylidene groups, probably due to the 'flip' of the cyclohexyl ring at normal NMR temperature which places the atom in a slightly different electronic environment; compare the two values (δ 22.75 and 22.54) for this atom in the monoprotected monosaccharide case (**20**).

The ^1H NMR spectrum of **19** showed a characteristic triplet at δ 0.85 (terminal methyl group), a large singlet at δ 1.22 (polymethylene peak), and a multiplet at δ 1.61 (masked by the broad cyclohexylidene peak) for the methylene group β to the carbonyl of the ester. At δ 2.36 was the expected multiplet for the methylene group adjacent to the carbonyl of the ester. Again, the cyclohexylidene acetal protons appear as two broad multiplets at δ 1.41 and 1.62.

The ^{13}C NMR spectrum of the triprotected cyclohexylidene monopalmitate **19** displayed a single, characteristic carbonyl shift at δ 173.0. Also observed was the characteristic shift pattern of the terminal methyl group and the three adjacent $-CH_2-$ groups. These appeared at shifts of δ 14.15, 22.70, 31.94, and 29.72, respectively. There was the usual poly-(CH_2)-complex at δ 29.40 $\pm$ 0.30. The two methylene groups adjacent to the ester carbonyl in the long chain were also clearly identifiable, appearing at δ 34.10 and 24.78. The result observed for the cyclohexylidene ring carbons was also seen in this compound.

This result and corresponding analytical data were in good correlation with our studies of monosaccharide models. Further reactions of the triprotected substrate **15** with other fatty acids have given the same, reproducible results.

Overall, it was possible to prepare a crystalline 'triprotected'-α,α'-trehalose (**15**) unit, for which regioselective esterification at the 2-position is possible, to afford the monoester **19**. This has excellent possibilities as a synthetic intermediate to permit regioselective synthesis of the 2,3-di-*O*-acyl-α,α'-trehaloses isolated from *M. tuberculosis* and *M. fortuitum* [11]. (The NMR data are given in Tables 1–4.)

3. Experimental

General methods.—Solvents were dried according to standard literature procedures. Melting points (uncorrected) were recorded using either a Kofler hot-stage apparatus or capilliaries and a Gallenkamp electrothermal machine. Elemental analyses of crystalline solids were obtained using a Carlo–Erba Instrumentazione model 1106 CHN analyser. IR spectra were recorded on a Nicolet 20 SXB or a Nicolet 20PC Fourier-Transform spectrometer; peaks are labelled br (broad), s (strong), m (medium), and w (weak). ^1H and ^{13}C NMR spectra (δ values, ppm) were obtained using solutions in $CDCl_3$ on a Bruker WP 200 or a Bruker AWX 500 instrument. Electron-impact mass spectra were recorded on a Kratos MS 80RF spectrometer; accurate masses were only available on molecular ions having masses $< m/z$ 600. Optical rotations were recorded on an NPL automatic polarimeter type 143D or on a PolAAr 2001 automatic polarimeter.

Starting materials and chemical reagents were purchased from Aldrich, Fluka, Fisons, and Lancaster Syntheses. Column chromatography was carried out at medium pressure using Merck 7736 grade silica gel. Fluka 60738 Silica Gel 60 or Fisons Matrix Silica 60 was used for flash column chromatography. Thin-layer chromatography (TLC) used Merck 5554 precoated silica-gel aluminium-backed sheets; all compounds were revealed by spraying with a 10% solution of molybdophosphoric acid (MPA) in EtOH followed by heating at 180°C for 15 min. A spray reagent of 5% solution of α-naphthol in aq ethanolic H_2SO_4, followed by heating at 110°C for 15 min, was used to detect carbohydrates [25]. Solvent system *A*, 1:1 petroleum ether (bp 40–60°C)–EtOAc; *B*, 2:1 petroleum ether (bp 40–60°C)–EtOAc.

*Methyl 2,3-di-*O*-acetyl-4,6-*O*-cyclohexylidene-α-*D*-glucopyranoside* (**20**) *[22].*—Methyl α-D-glucopyranoside (0.775 g, 3.429 mmol, 1.0 equiv) was dissolved in anhyd DMF (20 mL) and stirred over activated 4A molecular sieves under N_2. 1,1-Dimethoxycyclohexane (0.703 g, 4.88 mmol, 1.42 equiv) and *p*-toluenesulfonic acid (20 mg) were added and the mixture was stirred overnight at room temperature. The reaction was quenched by the addition of solid $NaHCO_3$ (1.223 g) and the mixture stirred for a further 1 h at room temperature. The mixture was filtered and the solvent removed in vacuo to yield a crude syrup. This was dissolved in dry pyridine (10 mL) and cooled to 0°C. A 1:1 mixture of dry pyridine (5 mL) and Ac_2O (5 mL) was added, the mixture stirred for 16 h at room temperature and then poured onto ice–water (50 mL), and the products extracted with $CHCl_3$ (3×40 mL). The combined organic extracts were washed with satd aq $CuSO_4$ (3×50 mL), satd aq NaCl (100 mL), and then dried over $MgSO_4$. The $CHCl_3$ solution

Table 1
^{1}H NMR chemical shifts (ppm) (200 MHz) for ring protons of α,α'-trehalose derivatives

Compound	PhC*H*	H-1 H-1′	H-2 H-2′	H-3 H-3′	H-4 H-4′	H-5 H-5′	H-6a H-6′a	H-6b H-6′b
1		5.22	4.89	5.39	[←	3.91–3.52		→]
		5.22	4.89	5.39	[←	3.91–3.52		→]
2		5.17	3.56	[3.84 4.02]^a	3.34	[3.84 4.02]^a	3.56	4.21
		5.17	3.56		3.34		3.56	4.21
3		5.22	4.90	5.47	3.41	3.47	3.79	3.95
		5.22	4.90	5.47	3.41	3.47	3.79	3.95
5	5.48	5.32	4.99	5.61	3.66	3.96	3.73	4.15
	5.48	5.32	4.99	5.61	3.66	3.96	3.73	4.15
6		5.22	4.91	5.41	[←	3.39–4.14		→]
		5.22	4.91	5.41	[←	3.39–4.14		→]
7		5.39	[←		3.04–3.46			→]
		5.39	[←		3.04–3.46			→]
8		5.19	4.94	5.45	3.42	3.48	[3.74–4.27]	
		5.30	4.96	5.48	3.42	3.48	[3.74–4.27]	
9		5.21	5.24	5.53	[←	3.12–4.25		→]
		5.34	5.24	4.87	[←	3.12–4.25		→]
10		5.25	5.01	5.45	5.01	[←	3.92–4.25	→]
		5.25	5.01	5.45	5.01	[←	3.92–4.25	→]
11		5.24	[4.84 5.01]^b	[5.32 5.40]^c	[←	3.39–4.24		→]
		5.29			[←	3.39–4.24		→]
12		[5.29 5.31]^d	4.91	5.33	[←	3.36–4.22		→]
			[←		3.36–4.22			→]
13		5.15	[←		3.31–4.11			→]
		5.39	[←		3.31–4.11			→]
14		5.27	4.89	5.35	[←	4.24–4.64		→]
		5.32	[←		3.64–4.24			→]
15		5.12	[←		3.42–3.82			→]
		5.38	[←		3.42–3.82			→]
16		5.39	[←		3.52–3.83			→]
		5.39	[←		3.52–3.83			→]
17		5.24	[3.33 3.63]^e	[3.89 4.14]^f	[3.33 3.63]^e	[3.89 4.14]^f	[3.33 3.63]^e	[3.89 4.14]^f
		4.99						
18	5.47	5.15	3.91–	4.45	3.54	3.54	[3.91–4.18]	
		5.15	4.18	4.45	3.54	3.54	[3.91–4.18]	
19		5.26	4.75	[←		3.39–4.09		→]
		5.34	[←		3.39–4.09			→]
25	5.47	5.17	3.85	4.48	g	h	g	h
	5.53	5.36	5.04	5.57	3.66	g	g	4.30

[a] H-3/3′ and H-5/5′ contained in this multiplet.
[b] H-2/2′ contained in this multiplet.
[c] H-3/3′ contained in this multiplet.
[d] H-1/1′ contained in this multiplet.
[e] H-2/2′, 4/4′, and 6a/6′a contained in this multiplet.
[f] H-3/3′, 5/5′, and 6b/6′b contained in this multiplet.
[g] Multiplet (4 H).
[h] Multiplet (2 H).

Table 2
^{1}H NMR data (ppm) (200 MHz) for ring protons of methyl α-D-glucopyranoside derivatives

Compound	PhC*H*	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
20		4.79	4.80	5.33	[←	3.46–3.84		→]
21		4.74	[←		3.42–3.88			→]
22		4.88	4.79	5.28	[←	3.45–3.84		→]
23	5.52	4.79	3.62	3.91	3.48	3.52	3.62	4.28
24	5.49	4.93	4.90	5.57	3.63	3.95	3.78	4.29

was filtered and the solvent removed in vacuo to yield a crude oil. This was purified using flash column chromatography (solvent *B*) to afford **20** as a colourless syrup (0.487 g, 36%). IR (film): 3059.49 (w, alkyl), 2939.89 (s, CH_3– and alkyl), 2860.80 (s, CH_3– and alkyl), 1747.73 cm^{-1} (s, C=O). Found M^+ 358.1691, $C_{17}H_{26}O_8$ requires 359.1901. ^{1}H NMR (200 MHz): 1.45 (m, br, 6 H, cyclohexyl), 1.60 (m, br, 4 H, cyclohexyl), 1.98 (s, 3 H, AcO-3), 2.02 (s, 3 H, AcO-2), 3.31 (s, 3 H, OMe), see Table 2 for ring protons; ^{13}C NMR (50.3 MHz): 20.77 (*C*H_3CO–O–2,3), 22.65 (cyclohexyl C-3), 22.75 (cyclohexyl C-4), 25.55 (cyclohexyl C-5), 27.57 (cyclohexyl C-6), 37.78 (cyclohexyl C-2), 55.20 (OCH_3), 99.82 (–O–C–O–, cyclohexyl), 169.82 (CH_3*C*O–O-3), 170.42 (CH_3*C*O–O-2); see Table 3 for ring carbons.

Methyl 4,6-O-cyclohexylidene-α-D-glucopyranoside (**21**) *[22].*—Compound **20** (102 mg, 0.285 mmol) was dissolved in $CHCl_3$ (5 mL). Sodium metal (200 mg) was dissolved in MeOH (5 mL) and added to the $CHCl_3$ solution, and the mixture was stirred for 30 min

Table 3
^{13}C NMR data (ppm) (50.3 MHz) for ring carbons of α,α′-trehalose and methyl α-D-glucopyranoside derivatives

Compound	C-1 C-1′	C-2 C-2′	C-3 C-3′	C-4 C-4′	C-5 C-5′	C-6 C-6′
6	92.80	71.30	70.83	64.13	69.47	61.42
	92.80	71.30	70.83	64.13	69.47	61.42
7	93.30	72.83	72.48	64.21	69.91	61.53
	93.30	72.83	72.48	64.21	69.91	61.53
8	93.21	78.55	76.44	63.11	70.64	61.77
	92.25	69.98	68.95	68.53	68.09	62.17
11	93.53	71.30	70.71	64.10	69.43	61.45
	92.73	70.50	70.06	68.95	68.95	62.17
14	93.39	71.30	71.14	64.06	69.46	61.49
	94.09	76.04	73.28	66.37	72.92	61.60
15	95.99	72.59	71.99	63.92	65.90	61.54
	93.98	76.19	73.28	66.41	73.02	61.54
16	94.41	76.30	73.51	72.99	66.04	61.60
	94.41	76.30	73.51	72.99	66.04	61.60
19	93.45	71.39	69.11	63.73	65.91	61.47
	93.88	76.08	73.36	66.29	72.99	61.47
20	97.62	71.56	71.35	69.50	65.83	61.71
25	92.91	69.91	69.84	68.51	68.18	61.71
	92.91	69.91	69.84	68.51	68.18	61.71

Table 4
Coupling constants (J Hz) for ring protons of α,α'-trehalose and methyl α-D-glucopyranoside derivatives

Compound	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$
1	4.0	8.8	9.2		4.0	8.8	9.2	
2	3.8				3.8			
3	4.0	9.9	9.8	9.5	4.0	9.9	9.8	9.5
5	4.1	9.9	9.7	9.4	4.1	9.9	9.7	9.4
6	4.0	8.9	9.3		4.1	8.9	9.3	
7	3.1				3.1			
8	4.0	9.4	9.4	9.5	4.0	10.0	9.8	10.0
9	3.6				3.5			
10	4.7	9.4	9.3	9.2	4.7	9.4	9.3	9.2
11	3.7	9.8			3.7	9.7		
12	3.1				3.9	9.9		
13	3.7				3.8			
14	3.0				3.8	9.8	9.8	
15	3.0				3.6			
16	3.0				3.0			
17	3.0				4.0	9.6		
18	3.9	9.0	9.0	9.4	3.9	9.0	9.0	9.4
19	3.0				3.7	9.6		
20	5.5	9.4	9.4					
21	3.9							
22	3.6	10.1	9.1					
23	3.9	9.5	9.3	9.2				
24	4.1	9.7	9.5	9.4				
25	3.9	8.8	8.8		4.0	9.9	9.8	9.9

at room temperature. The reaction was quenched by solid CO_2, followed by water (10 mL). The products were extracted using $CHCl_3$ (3 × 10 mL) and the combined organic extracts washed with satd aq NaCl (10 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to yield the product, which was purified by flash column chromatography (solvent *A*). The product was a thick, colourless oil (27.6 mg, 43%); IR (film): 3455.66 (s, br, OH), 3057.56 (m, alkyl), 2937.96 (s, alkyl), 2860.80 cm^{-1} (m, alkyl). Found M^+ 274.1422, $C_{13}H_{22}O_6$ requires 274.3132. ^1H NMR (200 MHz): 1.44 (m, br, 6 H, cyclohexyl), 1.60 (m, br, 4 H, cyclohexyl), 3.40 (s, 3 H, OMe); see Table 2 for ring protons.

Methyl 2,3-di-O-acetyl-α-D-glucopyranoside (**22**) *[26,27].*—Compound **20** (103 mg, 0.288 mmol) was dissolved in THF (2 mL) and aq 10% HCl (2 mL) was added. The solution was stirred at room temperature for 2 h before the addition of solid $NaHCO_3$ (2.213 g). Water (20 mL) and CH_2Cl_2 (15 mL) were added and the aqueous phase was extracted further with CH_2Cl_2 (3 × 15 mL). The organic extracts were washed with satd aq NaCl (30 mL), dried over $MgSO_4$, and filtered. The solvent was removed in vacuo to leave a homogeneous product, which was not further purified (57.9 mg, 74%); IR (film): 3435.66 (s, br, OH), 3057.56 (m, alkyl), 2937.96 (s, alkyl), 2860.80 (s, alkyl), 1734.23 cm^{-1} (s, C=O). Found M^+ 278.1796, $C_{11}H_{18}O_8$ requires 278.2584. ^1H NMR (200 MHz): 2.06 (s, 3 H, AcO-2), 2.07(s, 3 H, AcO-3); see Table 2 for ring protons.

Octa-O-acetyl-α,α′-trehalose (**10**) *and 2,3,2′,3′-tetra-O-acetyl 4,6:4′,6′-di-O-benzylidene-α,α′-trehalose* (**5**).—For the purposes of NMR spectroscopic comparison, the former compound was prepared by reaction of trehalose with acetic anhydride and pyridine, and the latter according to the procedure of Hanessian and Plessas [15].

2,3-Di-O-acetyl-4,6:2′,3′:4′,6′-tri-O-isopropylidene-α,α′-trehalose (**12**), *2,3,2′,3′-tetra-O-acetyl-4,6:4′,6′-di-O-isopropylidene-α,α′-trehalose* (**1**), *and 2,3,2′,3′,4′,6′-hexa-O-acetyl-4,6-O-isopropylidene-α,α′-trehalose* (**9**) *[13].*—Anhydrous α,α′-trehalose [12] (1.967 g, 5.756 mmol) was dissolved in anhyd DMF (30 mL), and *p*-toluenesulfonic acid (20 mg) and activated 4A molecular sieves were added under N_2. The solution was cooled to 0°C and 2-methoxypropene (1.724 g, 2.30 mL; 23.9 mmol, 4.1 equiv) was added. The mixture was stirred for 4 h at 0°C after which solid $NaHCO_3$ (3.021 g) was added with vigorous stirring for a further 1 h at room temperature. The resulting mixture was filtered and concentrated at 40°C in vacuo, to yield a crude yellow syrup which was dissolved in dry pyridine (15 mL) and cooled to 0°C. Dry pyridine (15 mL) and Ac_2O (15 mL) were mixed together and added to the solution which was allowed to warm to room temperature followed by stirring for 16 h. The solution was then poured onto ice–water (100 mL) and the products were extracted with $CHCl_3$ (3 × 100 mL). The combined organic layers were washed with satd aq $CuSO_4$ (3 × 100 mL), satd aq NaCl (100 mL), and then dried over $MgSO_4$. The mixture was filtered and the solution concentrated in vacuo to give a colourless syrup. Medium-pressure column chromatography, using petroleum ether (bp 40–60°C) and EtOAc (9:1 to 5:1 to 2:1 to 1:1 stepped gradient), gave **12** (R_f 0.38, TLC solvent *A*) (79.7 mg, 3%), then **1** (R_f 0.51) (1.051 g, 55%), and **9** (R_f 0.67) (246 mg, 12%), all as thick colourless syrups.

Compound **12** showed IR (film): 2988.12 (s, alkyl), 2924.46 (m, alkyl), 1757.37 cm^{-1} (s, C=O). Found: M^+ 531.1986, $C_{25}H_{37}O_{13}$ requires 545.5595, $C_{25}H_{37}O_{12}$ requires 529.5601; glycoside cleavage fragment *m/z* 287, $C_{13}H_{19}O_7$ requires 287.2889; glycoside cleavage fragment *m/z* 243, $C_{12}H_{19}O_5$ requires 243.2791. ^1H NMR (200 MHz): δ 1.33 (s, 3 H, -*Me*CMe–), 1.38 (s, 3 H, –MeC*Me*–), 1.43 (s, 6 H, –*Me*CMe–), 1.49 (s, 6 H, –MeC*Me*–), 2.05 (s, 3 H, AcO-3), 2.10 (s, 3 H, AcO-2); see Table 1 for ring protons.

Compound **1** showed IR (film): 2993.90 (m, alkyl), 2945.68 (m,alkyl), 2883.95 (m, alkyl), 1757.37 cm^{-1} (s, C=O). Found M^+ 575, $C_{26}H_{38}O_{15}$ requires 590.5787, $C_{26}H_{38}O_{14}$ requires 574.5778; glycoside cleavage fragment *m/z* 287, $C_{13}H_{19}O_7$ requires 287.2889. ^1H NMR (200 MHz): δ 1.35 (s, 6 H, –*Me*CMe–), 1.42 (s, 6 H, –MeC*Me*–), 2.04 (s, 6 H, AcO-3/3′), 2.11 (s, 6 H, AcO-2/2′); see Table 1 for ring protons.

Compound **9** showed IR (film): 2997.76 (m, alkyl), 2943.75 (m, alkyl), 1749.66 cm^{-1} (m, C=O). $C_{27}H_{38}O_{17}$ requires 634.5870, $C_{27}H_{38}O_{16}$ requires 618.5876; M 287, $C_{13}H_{19}O_7$ requires 287.2889; M 331, $C_{14}H_{19}O_9$ requires 331.2987. ^1H NMR (200 MHz): δ 1.31 (s, 3 H, –*Me*CMe–), 1.39 (s, 3 H, –MeC*Me*–), 1.98 (s, 3 H, AcO-6), 2.01 (s, 3 H, AcO-4), 2.08 (s, 6 H, AcO-3/3′), 2.11 (s, 6 H, AcO-2/2′); see Table 1 for ring protons.

2,3:4,6:2′,3′:4′,6′-Tri-O-isopropylidene-α,α′-trehalose (**13**).—Sodium (50 mg) was dissolved in MeOH (2 mL) and the solution added to a solution of **12** (13.7 mg, 25 μmol) in $CHCl_3$ (5 mL). The resulting solution was stirred for 30 min, before quenching with solid CO_2. Ice–water (10 mL) was added and the products were extracted with $CHCl_3$ (3 × 5 mL). The organic layers were washed with satd aq NaCl (10 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to yield **13** as a colourless oil (9.1 mg, 79%),

which was homogeneous in TLC (R_f 0.38 in solvent *A*). IR (film): 3445.30 (s, br, OH), 2918.67 (m, alkyl), 2851.15 cm^{-1} (m, alkyl). Found M^+ 463, $C_{21}H_{34}O_{11}$ requires 462.4930; M 243, $C_{12}H_{19}O_5$ requires 243.2791; glycoside cleavage fragment *m/z* 203, $C_9H_{15}O_5$ requires 203.2145. ^1H NMR (200 MHz): δ 1.42 (s, 6 H, –*Me*CMe–), 1.45 (s, 6 H, –MeC*Me*–), 1.49 (s, 3 H, –*Me*CMe–), 1.53 (s, 3 H, –MeC*Me*–); see Table 1 for ring protons.

4,6:4',6'-Di-O-isopropylidene-α,α'-trehalose (**2**).—The title compound was prepared from **1** by deacylation [13], as described above.

2,3:2',3'-Di-O-(1,3-dimethyl-2-oxa-1,3-propanediyl)-4,6:4',6'-di-O-ethylidene-α,α'-trehalose (**17**), *2,3,2',3'-tetra-O-acetyl-4,6:4',6'-di-O-ethylidene-α,α'-trehalose* (**3**), *and 2,3,2',3',4',6'-hexa-O-acetyl-4,6-O-ethylidene-α,α'-trehalose* (**8**) *[14].*—Anhydrous α,α'-trehalose (1.667 g, 4.875 mmol) was dissolved in acetaldehyde (23.64 g, 30.0 mL, 0.537 mol) under N_2 and stirred over activated 4A molecular sieves at room temperature. Concentrated H_2SO_4 (0.4 mL) was added to the mixture and the solution stirred for 1.5 h at room temperature before the addition of solid $NaHCO_3$ (4.022 g) as quenching agent. The mixture was stirred for a further 1 h at room temperature, filtered, and concentrated in vacuo to yield a viscous oil. A solution of the crude product in dry pyridine (20 mL) was cooled to 0°C, and a 1:1 mixture of dry pyridine (10 mL) and Ac_2O (10 mL) added, and the solution stirred for 16 h at room temperature. The resulting golden solution was poured onto ice–water (50 mL) and the products were extracted using $CHCl_3$ (4×50 mL). The combined organic layers were washed sequentially with satd aq $CuSO_4$ (3×100 mL) and satd aq NaCl (100 mL), and dried over $MgSO_4$. The $CHCl_3$ solution was filtered and the solvent removed in vacuo to yield the crude products. Flash column chromatography with petroleum ether (bp 40–60°C) and EtOAc (4:1 to 2:1 gradient) gave **17** (0.876 g, 34%; syrup, R_f 0.71 in solvent *A*), then **3** (0.772 g, 28%; mp 161–163°C; R_f 0.59), and **8** (46.5 mg, 2%; syrup; R_f 0.43).

Compound **17** showed IR (film): 2988.12 (s, alkyl), 2939.89 (s, alkyl), 2893.59 (s, alkyl), 2876.23 cm^{-1} (s, alkyl). Found M^+ 533, $C_{24}H_{38}O_{13}$ requires 534.5564; glycoside cleavage fragment *m/z* 259, $C_{12}H_{19}O_6$ requires 259.2785. ^1H NMR (200 MHz): δ 1.30–1.39 (m, 18 H, –*Me*CH–), 4.68–4.74 (m, 6 H, –MeC*H*–); see Table 1 for ring protons. Found: C, 53.95; H, 7.15%. $C_{24}H_{38}O_{13}$ requires: C, 53.93; H, 7.16%.

Compound **3** showed $[\alpha]_D^{21}$ +168.0° (*c* 0.5, $CHCl_3$); IR (film): 2947.61 (m, alkyl), 1755.45 cm^{-1} (s, C=O); Found M^+ 562, $C_{24}H_{34}O_{15}$ requires 562.5236; glycoside cleavage fragment *m/z* 273, $C_{12}H_{17}O_7$ requires 273.2621. ^1H NMR (200 MHz): δ 1.30 (d, 6 H, *J* 5.0 Hz, *Me*CH–), 2.07 (s, 6 H, AcO-3/3'), 2.12 (s, 6 H, AcO-2/2'); see Table 1 for ring protons. Found: C, 51.06; H, 6.12%. $C_{24}H_{34}O_{15}$ requires: C, 51.29; H, 6.10%.

Compound **8** showed IR (film): 2963.04 (w, alkyl), 2856.94 (w, alkyl), 1755.45 cm^{-1} (s, C=O). Found M^+ 620, $C_{26}H_{36}O_{17}$ requires 620.5602; glycoside cleavage fragment *m/z* 331, $C_{14}H_{19}O_9$ requires 331.2987; glycoside cleavage fragment *m/z* 273, $C_{12}H_{17}O_7$ requires 273.2621. ^1H NMR (200 MHz): δ 1.30 (d, 3 H, *J* 5.0 Hz, *Me*CH–), 2.11 (s, 3 H, AcO-6), 2.05 (s, 3 H, AcO-4), 2.03 (s, 6 H, AcO-3/3'), 2.01 (s, 6 H, AcO-2/2'), 4.64 (q, 1 H, *J* 5.0 Hz, MeC*H*–); see Table 1 for ring protons. ^{13}C NMR (50.3 MHz): 20.22 (CH_3CO–O-2), 20.64 (CH_3CO–O-3), 20.88 (CH_3CO–O-4/4'), 20.94 (CH_3CO–O-6/6'), 22.71 (–CH_3–CH), 99.942 (–CH_3–CH), 169.70 (CH_3CO–O-6), 169.86 (CH_3CO–O-4), 169.98 (CH_3CO-O-3/3'), 170.61 (CH_3CO–O-2/2'); see Table 3 for ring carbons.

The title compounds were also prepared according to the procedure of Birch [14], using paraldehyde and H_2SO_4 catalysis. Purification using flash column chromatography with petroleum ether (bp 40–60°C) and EtOAc (3:1 to 2:1 to 1:1 gradient) gave **17** (0.430 g, 18%), then **3** (1.405 g, 56%), and **8** (0.332 g, 12%).

4,6:4',6'-Di-O-*benzylidene-2,3:2'3'-di*-O-(*tetraphenyldisiloxane-1,3-diyl*)-α,α'-*trehalose* (**18**) *and 2,3-di*-O-*acetyl-4,6:4',6'-di*-O-*benzylidene-2',3'-*(*tetraphenyldisiloxane-1,3-diyl*)-α,α'-*trehalose* (**25**).—Compound **4** (0.746 g, 1.474 mmol) was dissolved in dry CH_2Cl_2 (50 mL) and anhyd DMF (5 mL) with stirring over activated 4A molecular sieves. Imidazole (0.442 g, 6.49 mmol, 4.4 equiv) was added and the solution stirred at room temperature for 5 min. Dichlorodiphenylsilane (0.34 g, 0.30 mL, 1.474 mmol, 1.0 equiv) was added and the mixture stirred for 16 h at room temperature. The reaction was quenched by the addition of satd aq NH_4Cl (50 mL) and the organic products were extracted using $CHCl_3$ (3×40 mL). The combined organic layers were washed with satd aq NaCl (80 mL) and then dried over $MgSO_4$. The solvent was removed in vacuo and the crude products were dissolved in dry pyridine (10 mL) with cooling to 0°C. Dry pyridine (5 mL) and Ac_2O (5 mL) were added and the solution was stirred at room temperature for 14 h. The solution was poured onto ice–water (50 mL) and the products were extracted with $CHCl_3$ (3×40 mL). The $CHCl_3$ layers were washed sequentially with satd aq $CuSO_4$ (3×50 mL) and satd aq NaCl (100 mL), and then dried over $MgSO_4$. The solvent was removed in vacuo to yield a crude oil which was purified by flash column chromatography using petroleum ether (bp 40–60°C) and EtOAc (3:1 to 2:1 to 1:1 gradient) to yield **18** (0.192 g, 12%, R_f 0.98 in solvent *A*); **25** (0.1062 g, 9%, R_f 0.93 in solvent *A*), and **5** (0.3221 g, 40%, R_f 0.75 in solvent *A*).

Compound **18** showed IR (film): 3136.65 (w, br, aryl), 3071.07 (s, aryl), 3051.77 (s, aryl), 3022.84 (s, aryl), 3003.55 (s, aryl), 2930.24 cm^{-1} (m, C–H). Found M^+ none detected, $C_{74}H_{66}O_{13}Si_4$ requires 1275.6716; glycoside cleavage fragment *m/z* 629, $C_{37}H_{33}O_6Si_2$ requires 629.8361. ^1H NMR (200 MHz): 7.02–7.91 (cm, 40 H, *Ph*CH– and Ph_2Si–); see Table 1 for ring protons.

Compound **25** showed: Found M^+ 1039, $C_{54}H_{52}O_6Si_4$ requires 1037.3404. ^1H NMR (200 MHz): 2.05 (s, 3 H, AcO-3), 2.09 (s, 3 H, AcO-2), 7.39–7.55 (m, 18 H, Ph); see Table 1 for ring protons.

Compound **5** showed: IR (KBr disc): 1753.52 cm^{-1} (s, C=O). ^1H NMR (200 MHz): 2.06 (s, 6 H, AcO-3,3'), 2.12 (s, 6 H, AcO-2/2'), 7.32–7.35 (m, 6 H, *Ph*CH–), 7.40–7.43 (m, 4 H, *Ph*CH–); see Table 1 for ring protons.

2,3:4,6:2',3':4',6'-Tetra-O-*cyclohexylidene-α,α'-trehalose* (**16**), *2,3-di*-O-*acetyl-4,6:2',3':4',6'-tri*-O-*cyclohexylidene-α,α'-trehalose* (**14**), *2,3,2',3'-tetra*-O-*acetyl-4,6:4',6'-di*-O-*cyclohexylidene-α,α'-trehalose* (**6**), *and 2,3,4,6,2',3'-hexa*-O-*acetyl-4',6'*-O-*cyclohexylidene-α,α'-trehalose* (**11**).—Anhydrous α,α'-trehalose (2.760 g, 8.07 mmol) was dissolved in anhyd DMF (50 mL) and stirred over activated 4A molecular sieves. *p*-Toluenesulfonic acid (20 mg) and 1,1-dimethoxycyclohexane [23] (9.595 g, 66.6 mmol, 8.1 equiv) were added, and the mixture placed under reduced pressure (water pump) and heated to reflux. A steady reflux was maintained for 4 h before the mixture was cooled and solid $NaHCO_3$ (4.35 g) added to quench the reaction. The mixture was filtered and the solvent removed in vacuo. The resulting crude syrup was dissolved in dry pyridine (20 mL), the mixture cooled to 0°C, and a mixture of dry pyridine (10 mL) and Ac_2O (10

mL) added. This solution was stirred for 16 h and then poured onto ice–water (100 mL). The products were extracted using $CHCl_3$ (3 × 75 mL), and the organic layers washed with satd aq $CuSO_4$ (3 × 100 mL), satd aq NaCl (150 mL), and dried over anhyd $MgSO_4$. The $CHCl_3$ extracts were filtered and the solvent removed in vacuo to yield a crude syrup. Purification by flash column chromatography using petroleum ether (bp 40–60°C) and EtOAc (4:1 to 2:1 gradient) gave **16** (0.163 g, 3%), then **14** (2.268 g, 42%), **6** (2.091 g, 39%), and **11** (0.329 g, 6%).

Compound **16** was a colourless syrup, R_f 0.86 in solvent *A*; $[\alpha]_D^{21}$ +87.2° (*c* 1, $CHCl_3$); IR (film): 3057.56 (w, alkyl), 2937.96 (s, alkyl), 2860.80 cm^{-1} (s, alkyl). Found M^+ 662.3437, $C_{36}H_{54}O_{11}$ requires 662.8160; glycoside cleavage fragment *m/z* 323, $C_{18}H_{27}O_5$ requires 323.4083. ^1H NMR (200 MHz): 1.49 (m, br, 24 H, cyclohexyl), 1.63 (m, br, 16 H, cyclohexyl); see Table 1 for ring protons; ^{13}C NMR (50.3 MHz): 22.57, 23.56 (cyclohexyl C-4), 23.56, 23.85, 25.06, 25.62 (cyclohexyl C-3,5), 36.08, 36.28, 37.79, 37.82 (cyclohexyl C-2,4), 99.75 (cyclohexyl C-1, across 4,6 and 4′,6′), 112.62 (cyclohexyl C-1, across 2,3 and 2′,3′); see Table 3 for ring carbons.

Compound **14** was a white solid, R_f 0.63 in solvent *A*; mp 90–94°C; $[\alpha]_D^{21}$ +108.0° (*c* 1, $CHCl_3$); IR (KBr disc): 2939.89 (s, alkyl), 2860.80 (m, alkyl), 1751.59 cm^{-1} (s, C=O). Found M^+ 666, $C_{35}H_{50}O_{13}$ requires 666.7612; glycoside cleavage fragment *m/z* 327, $C_{17}H_{23}O_7$ requires 327.3535; glycoside cleavage fragment *m/z* 323, $C_{18}H_{27}O_5$ requires 323.4083. ^1H NMR (200 MHz): 1.49 (m, br, 18 H, cyclohexyl), 1.63 (m, br, 12 H, cyclohexyl), 2.03 (s, 3 H, AcO-3), 2.09 (s, 3 H, AcO-2); see Table 1 for ring protons; ^{13}C NMR (50.3 MHz): 20.65 (CH_3CO–O-3), 20.81 (CH_3CO–O-2), 22.54, 22.72, 22.83 (cyclohexyl C-4), 23.55, 23.88, 25.05, 25.58 (cyclohexyl C-3,5), 35.92, 36.30, 37.78, 37.85 (cyclohexyl C-2,6), 99.45 (cyclohexyl C-1, across 4,6), 99.90 (cyclohexyl C-1, across 4′,6′), 112.60 (cyclohexyl C-1, across 2′,3′), 169.73 (CH_3CO–O-3), 170.35 (CH_3CO–O-2); see Table 3 for ring carbons. Found: C, 61.39; H, 7.62%; $C_{35}H_{50}O_{13}$ requires: C, 61.25; H, 7.56%.

Compound **6** was a colourless syrup, R_f 0.38 in solvent *A*; $[\alpha]_D^{21}$ +122.4° (*c* 1, $CHCl_3$); IR (film): 3059.49 (w, alkyl), 2939.89 (s, alkyl), 2862.73 (m, alkyl), 1751.59 cm^{-1} (s, C=O). Found M^+ 670, $C_{34}H_{46}O_{15}$ requires 670.7064; glycoside cleavage fragment *m/z* 327, $C_{17}H_{23}O_7$ requires 327.3535. ^1H NMR (200 MHz): 1.48 (m, br, 12 H, cyclohexyl), 1.61 (m, br, 8 H, cyclohexyl), 2.05 (s, 6 H, AcO-3/3′), 2.12 (s, 6 H, AcO-2/2′); see Table 1 for ring protons; ^{13}C NMR (50.3 MHz): 20.66, 20.81 (CH_3CO–O-2/2′/3/3′), 22.55, 22.74 (cyclohexyl C-4), 23.57, 23.91, 25.07, 25.60 (cyclohexyl C-3,5), 37.78 (cyclohexyl C-2,6), 99.94 (cyclohexyl C-1), 169.51 (CH_3CO–O-3/3′), 170.426 (CH_3CO–O-2/2′); see Table 3 for ring carbons.

Compound **11** was a colourless syrup, R_f 0.25 in solvent *A*; $[\alpha]_D^{21}$ +128.0° (*c* 1, $CHCl_3$); IR (film): 3059.49 (s, alkyl), 2937.96 (s, alkyl), 2860.80 (s, alkyl), 1749.66 cm^{-1} (s, C=O). Found M^+ 674, $C_{31}H_{42}O_{17}$ requires 674.6546; glycoside cleavage fragment *m/z* 331, $C_{17}H_{23}O_7$ requires 331.2987; glycoside cleavage fragment *m/z* 327, $C_{14}H_{19}O_9$ requires 327.3535. ^1H NMR (200 MHz): 1.48 (m, br, 4 H, cyclohexyl), 1.60 (m, br, 6 H, cyclohexyl), 2.00 (s, 3 H, AcO-6), 2.03 (s, 3 H, AcO-4), 2.04 (s, 6 H, AcO-3/3′), 2.10 (s, 6 H, AcO-2/2′); see Table 1 for ring protons; ^{13}C NMR (50.3 MHz): 20.73 (CH_3CO–O-4/6), 20.84 (CH_3CO–O-3/3′), 21.00 (CH_3CO–O-2/2′), 22.55, 22.75 (cyclohexyl C-4), 25.54, 27.53 (cyclohexyl C-3,5), 36.23, 37.78 (cyclohexyl C-2,6), 99.79 (cyclohexyl C-1),

169.69 (CH_3CO–O-6), 169.99 (CH_3CO–O-4), 170.25 (CH_3CO–O-3/3'), 170.26 (CH_3CO–O-2/2'); see Table 3 for ring carbons.

4,6:2',3':4',6'-Tri-O-*cyclohexylidene-α,α'-trehalose* (**15**).—Compound **14** (2.268 g, 3.405 mmol) was deacetylated as described previously. Purification by flash column chromatography, using petroleum ether (bp 40–60°C) and EtOAc (1:1 to 100% EtOAc), afforded **15** as a white solid (1.268 g, 64%); mp 138–140°C; $[\alpha]_D$ +112.0° [21] (*c* 1, $CHCl_3$); IR (KBr disc): 3456.88 (m, br, OH), 3055.63 (w, alkyl), 2937.96 (s, alkyl), 2860.80 cm^{-1} (s, alkyl). Found M^+ 582.4883, $C_{30}H_{46}O_{11}$ requires 582.6868; glycoside cleavage fragment *m/z* 323.1758, $C_{18}H_{27}O_5$ requires 323.4083; glycoside cleavage fragment *m/z* 243.1111, $C_{12}H_{19}O_5$ requires 243.2791. ^1H NMR (200 MHz): 1.22 (m, br, 18 H, cyclohexyl), 1.62 (m, br, 12 H, cyclohexyl), 2.31 (s, br, 1 H, OH), 2.73 (s, br, 1 H, OH); see Table 1 for ring protons; ^{13}C NMR (50.3 MHz): 22.54, 22.71, 22.82 (cyclohexyl C-4), 23.53, 23.86, 25.03, 25.59 (cyclohexyl C-3,5), 36.05, 36.24, 37.77, 37.89 (cyclohexyl C-2,6), 99.77 (cyclohexyl C-1, across 4,6), 100.00 (cyclohexyl C-1, across 4',6'), 112.50 (cyclohexyl C-1, across 2',3'); see Table 3 for ring carbons. Found: C, 61.48; H, 7.81%. $C_{30}H_{46}O_{11}$ requires: C, 61.84; H, 7.96%.

4,6:4',6'-Di-O-*cyclohexylidene-α,α'-trehalose* (**7**).—Compound **6** (3.924 g, 5.927 mmol) was subjected to deacetylation as described above. Purification by flash column chromatography, using petroleum ether (bp 40–60°C) and EtOAc (1:1 to 100% EtOAc), afforded **7** as a white solid (2.081 g, 73%); mp 104–108°C; $[\alpha]_D$ +91.6° [21] (*c* 1, $CHCl_3$); IR (KBr disc): 3458.81 (s, br, OH), 2937.96 (s, alkyl), 2862.73 cm^{-1} (m, alkyl). Found M^+ 502.2440, $C_{24}H_{38}O_{11}$ requires 502.5576; glycoside cleavage fragment *m/z* 243.1200, $C_{12}H_{19}O_5$ requires 243.2791. ^1H NMR (200 MHz): 1.23 (m, br, 12 H, cyclohexyl), 1.63 (m, br, 4 H, cyclohexyl), 2.32 (s, br, 1 H, OH), 2.74 (s, br, 1 H, OH); see Table 1 for ring protons; ^{13}C NMR (50.3 MHz): 22.49, 22.80 (cyclohexyl C-4), 25.3, 25.61, 27.05, 27.75 (cyclohexyl C-3,5), 37.75 (cyclohexyl C-2,6), 100.15 (cyclohexyl C-1); see Table 3 for ring carbons.

4,6:2',3':4',6'-Tri-O-*cyclohexylidene-α,α'-trehalose 2-palmitate* (**19**).—Compound **15** (0.3045 g, 0.523 mmol) was dissolved in dry CH_2Cl_2 (20 mL) containing activated 4A molecular sieves under N_2. Palmitic acid (0.133 g, 0.521 mmol, 1.0 equiv) and 4-dimethylaminopyridine (66.2 mg, 0.542 mmol, 1.05 equiv) were added and the solution was cooled to 0°C. Dicyclohexylcarbodiimide (0.137 g, 0.621 mmol, 1.2 equiv) was added and the mixture maintained at 0°C for 10 min before being allowed to warm to room temperature. Stirring was maintained for 4 h, with the formation of a white precipitate. This was filtered off and washed with cold CH_2Cl_2 (3 × 20 mL). The solvent was removed in vacuo to yield an off-white solid, which was purified by flash column chromatography, using 9:1 petroleum ether (bp 40–60°C)–EtOAc, to afford **19** as a white solid (0.323 g, 75%); mp 126–128°C; $[\alpha]_D^{21}$ +80.4° (*c* 1, $CHCl_3$); IR (film): 3422.15 (br, w, OH), 2928.32 (s, alkyl), 2855.01 (s, alkyl), 1747.73 cm^{-1} (s, C=O). Found M^+ 821, $C_{46}H_{76}O_{12}$ requires 821.0991; glycoside cleavage fragment *m/z* 481, $C_{28}H_{49}O_6$ requires 481.6915; glycoside cleavage fragment *m/z* 323, $C_{18}H_{27}O_5$ requires 323.4083; glycoside cleavage fragment *m/z* 339, $C_{18}H_{27}O_6$ requires 339.4077; straight-chain acid fragment *m/z* 239, $C_{16}H_{31}O$ requires 239.4203. ^1H NMR (200 MHz): 0.85 (t, 3 H, *J* 6.7 Hz, $CH_3CH_2CH_2$), 1.22 [s, 24 H, $-(CH_2)-$], 1.42 [m, br, 18 H, $-(CH_2)_3CH_2-C-(O)_2-$], 1.61 (m, 2 H, $-CH_2CH_2-CO-O-$), 1.64 [m, br, 12 H, $-(CH_2)_3-CH_2-C-(O)_2-$], 2.36 (m, 2 H, $-CH_2CH_2CO-O-$); see Table 1 for ring

protons; ^{13}C NMR (50.3 MHz): 14.15 ($CH_3CH_2CH_2CH_2$–), 22.53, 22.72, 22.82, 23.52, 23.82 (cyclohexyl C-4), 22.70 ($CH_3CH_2CH_2CH_2$–), 24.74, 24.84, 25.03, 25.47, 25.59 (cyclohexyl C-3,5), 24.78 (–CH_2CH_2CO–O–), 29.18, 29.26, 29.40, 29.63 [–(CH_2)–], 31.94 (–CH_2CH_2CO–O–), 34.10 ($CH_3CH_2CH_2CH_2$–), 34.96, 36.02, 36.24, 37.92 (cyclohexyl C-2,6), 99.83, 99.86 (cyclohexyl C-1, across 4,6 and 4′,6′), 112.46 (cyclohexyl C-1, across 2′,3′), 173.44 (C=O); see Table 3 for ring carbons.

Acknowledgements

This work was supported by a Science and Engineering Research Council (S.E.R.C.)-QUOTA award to P.A.W. The authors give special thanks to Dr. N.A. Hughes for many valuable discussions. They also thank Mr. I. McKeag (500-MHz NMR and 500-MHz COSY spectra), Mrs. L. Cook (200-MHz NMR spectra), Mr. D. Dunbar (FTIR spectra and microanalyses), and Mr. S. Addison (mass spectra).

References

[1] B.R. Bloom and C.J.C. Murry, *Science*, 257 (1992) 1055.
[2] D.E. Minnikin, G. Dobson, D. Sesardic, and M. Ridell, *J. Gen. Microbiol.*, 131 (1985) 1369–1374.
[3] G.S. Besra, R.C. Bolton, M.R. McNeil, M. Ridell, K.E. Simpson, J, Glushka, H. van Halbeek, P.J. Brennan, and D.E. Minnikin, *Biochemistry*, 31 (1992) 9832–9837.
[4] A. Lemassu, M.-A. Lanéelle, and M. Daffé, *FEMS Microbiol. Lett.*, 78 (1991) 171–176.
[5] H.H. Baer, *Carbohydr. Res.*, 240 (1993) 1–22.
[6] N. Gautier, L.M. López-Marín, M.-A. Lanéelle, and M. Daffé, *FEMS Microbiol. Lett.*, 98 (1992) 81–88.
[7] M.E. Hamid, J.L. Fraser, P.A. Wallace, G.S. Besra, M. Goodfellow, D.E. Minnikin, and M. Ridell, *Lett. Appl. Microbiol.*, 16 (1993) 132–135.
[8] M.A. Sempere, P.L. Valero-Guillén, A. de Godos, and F. Martin-Luengo, *J. Gen. Microbiol.*, 139 (1993) 585–590.
[9] H.H. Baer and X. Wu, *Carbohydr. Res.*, 238 (1993) 215–230.
[10] P.A. Wallace and D.E. Minnikin, *J. Chem. Soc., Chem. Commun.*, (1993) 1292–1293.
[11] P.A. Wallace and D.E. Minnikin, *J. Chem. Soc., Chem. Commun.*, (1994) 329–330.
[12] G. Birch, *J. Chem. Soc.*, (1965) 3489–3490.
[13] J. Defaye, H. Driguez, B. Henrissat, J. Gelas, and E. Bar-Guilloux, *Carbohydr. Res.*, 63 (1978) 41–49.
[14] G.G. Birch, *J. Chem. Soc.*, (1966) 1072–1074.
[15] S. Hanessian and N.R. Plessas, *J. Org. Chem.*, 34 (1969) 1035–1039.
[16] K. Freudenberg, H. Toepffer, and C.C. Andersen, *Ber.*, 61 (1928) 1727–1731.
[17] B.M. Trost and C.G. Caldwell, *Tetrahedron Lett.*, 22 (1981) 4999–5002.
[18] I. Paterson and M.V. Perkins, *Tetrahedron Lett.*, 33 (1992) 801–804.
[19] S. Anwar and A.P. Davis, *J. Chem. Soc., Chem. Commun.*, (1986) 831–832.
[20] W.T. Markiewicz, *J. Chem. Res. (M)*, (1979) 191; *J. Chem. Res. (S)*, (1979) 24.
[21] T. Ziegler, K. Neumann, E. Eckhardt, G. Herold, and G. Pantowski, *Synlett (S)*, (1991) 699–701.
[22] F.H. Bissett, M.E. Evans, and F.W. Parrish, *Carbohydr. Res.*, 5 (1967) 184–193.
[23] T. Tsumoda, M. Suzuki, and R. Noyori, *Tetrahedron Lett.*, 21 (1980) 1357–1358.
[24] B. Neises and W. Steglich, *Angew. Chem. Int. Ed. Engl.*, 17 (1978) 522–524.
[25] H. Jacin and A.R. Mishkin, *J. Chromatogr.*, 18 (1965) 170–172.
[26] J. Hill, L. Hough, and A.C. Richardson, *Carbohydr. Res.*, 8 (1968) 7–18.
[27] T.G. Bonner, *Methods Carbohydr. Chem.*, 2 (1963) 309–313.
[28] T.B. Grindley, C.J.P. Cote, and C. Wickramage, *Carbohydr. Res.*, 140 (1985) 215–238.