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Synthesis of L-trehalose and observations on isomer and by-product formation

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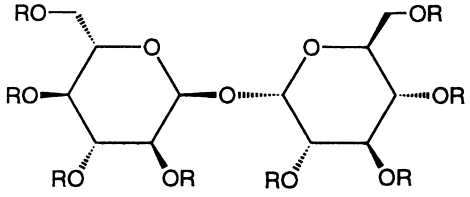
Abstract

With a view to gaining evidence on the mechanism by which D-trehalose is able to stabilise biomolecules towards dehydration (anhydrobiosis) and heat, L-trehalose has been prepared in order to allow comparative studies to be made. Little change can be induced in the ratio of the α,α-, α,β-, β,β-1,1′-stereoisomers of the disaccharide formed from 2,3,4,6-tetra-*O*-benzyl-L-glucose by using different reaction procedures and by varying the reaction conditions. Benzyl 2,3,4,6-tetra-*O*-benzyl α- and β-L-glucopyranoside are by-products in the trimethylsilyl trifluoromethanesulphonate mediated formation of the 1,1′-linked disaccharides.

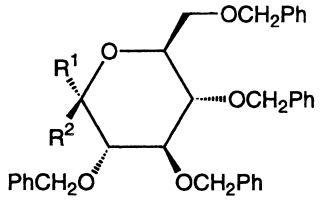
Keywords: L-Trehalose; Anhydrobiosis; Biostabilisation; 1,1′-Linked disaccharide

1. Introduction

Trehalose (α-D-glucopyranosyl α-D-glucopyranoside) occurs in many organisms that have the ability to survive heat and dehydration, for example some desert-living plants ('resurrection plants'), certain brine shrimps, some nematodes found in soil and dry Baker's yeast.[1] The concentration of trehalose in such dehydrated organisms, which are said to be in a state of 'anhydrobiosis', can be as high as 20% of their dry weight. Even after years in a dehydrated state, metabolic activity restarts on rehydration. The manner in which this disaccharide brings about stabilisation of biological molecules is still open to debate, although the two major hypotheses that have been advanced are based on: (i) the replacement of hydrogen bonding interactions with water molecules crucial to the integrity of the molecular architecture of important biomolecules and structures such as proteins, phospholipids bilayers, and membranes by related bonding to



1 R = H
3 R = CH_2Ph
8 R = Ac



	R^1	R^2
2	H	OH
6	OCH_2Ph	H
7	H	OCH_2Ph

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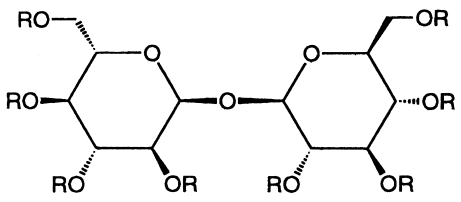
the sugar;[2] (ii) the formation of an inert glass by the disaccharide leading to the entrapment of key water molecules within and at the surface of the biomolecules ensuring their survival in a viable form on full rehydration.[3]



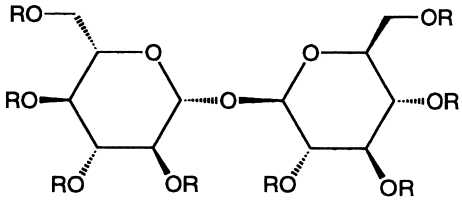
4 R = CH_2Ph

In view of the fact that specific carbohydrate-biomolecule interactions of the type involved in hypothesis (i) involve a form of recognition between two chiral environments (especially in the case of proteins), they should be sensitive to a change in the chirality of the disaccharide. This would not be expected to be the case for hypothesis (ii), in which only the glassy nature of the dehydrated disaccharide, forming a physical barrier to water removal, would seem to be involved. We consider, therefore, that experiments in which the abilities of D-trehalose and L-trehalose to bring about protection of biomolecules to heat and/or dehydration are compared (e.g., stabilisation of sensitive enzymes to dehydration[4]) would afford an insight into the mechanism by which the natural disaccharide exerts its protecting effects on organisms.

This paper describes a convenient synthesis of L-trehalose, and draws attention to certain aspects previously unreported in related syntheses of the D-isomer.

2. Results and discussion

A number of syntheses of D-trehalose (α-D-glucopyranosyl α-D-glucopyranoside) or its derivatives have been reported,[5] mechanistic considerations requiring a non-participating group at O-2 in order to avoid overriding formation of a β-linkage at the anomeric centre of the donor. 2,3,4,6-Tetra-*O*-benzyl-D-glucose has proved a convenient starting material, used as a donor molecule after conversion into the corresponding chloride,[5b,5c,5j] trichloroacetimidate,[5l] trimethylsilyl glycoside,[5c,5i] or used directly in a Lewis acid[5e,5k,5m] or acid anhydride[5g,5h] mediated process, presumably leading to an oxa-carbenium ion which is captured by an excess of the hemiacetal. In such syntheses a mixture of α,α-, α,β-, and β,β-isomers of the octa-*O*-benzyl-D-disaccharide are expected with product ratios depending on reaction conditions (e.g., solvent, temperature) and the anomeric composition (α,β ratio) of the acceptor and some authors have specified conditions which favour the α,α-D-isomer. In view of the relative scarcity of L-glucose, it was decided that as direct a route as possible should be used to prepare L-trehalose (**1**), in which a predominance of the α,α-isomer had been demonstrated in the D-series.[5m] This report describes



5 R = CH_2Ph

the related preparation of L-trehalose (**1**) but with the hitherto unreported observation of unexpected by-products formed in this procedure; however, despite careful experimentation, we have been unable to substantiate the reported[5m] preferential formation of the α,α-linked disaccharide.

2,3,4,6-Tetra-*O*-benzyl-L-glucose (**2**), a compound only described previously in a patent,[6] was prepared following essentially a route described[7] for the D-isomer with the important modification that isomerisation of allyl 2,3,4,6-tetra-*O*-benzyl-α,β-L-glucose was performed in dimethyl sulphoxide–toluene rather than in dimethyl sulphoxide alone, a useful modification introduced for the allyl to *cis*-1-propenyl ether isomerisation by Vanbaelinghem and co-workers.[8] The apparently preferred synthesis of D-trehalose requires reaction of 2,3,4,6-tetra-*O*-benzyl-D-glucose with trimethylsilyl trifluoromethanesulphonate in dichloromethane in the presence of a special molecular sieve, Davison SP 7-8461, which was claimed[5m] to be exceptional compared to other sieves in favouring formation of the α,α-linkage; isolated yields of α,α-, α,β-, and β,β-isomers of the octabenzyl ether were 49, 17, and 2.5%. Extensive enquiries, necessitated by the fact that the sieve is not listed under its original designation in chemical catalogues, led to its identification[†] as the currently marketed SYLOSIV® A4.

Reaction of 2,3,4,6-tetra-*O*-benzyl-L-glucose (**2**) with trimethylsilyl trifluoromethanesulphonate in dichloromethane in the presence of SYLOSIV® A4 did indeed give the α,α-isomer, octa-*O*-benzyl-L-trehalose (**3**), isolated by PLC, but only as the minor product, yields of between 24 and 27% being consistently obtained in repeated experiments. The efficient separation of the three stereoisomeric products is extremely difficult, especially so the separation of the α,β- and β,β-isomers, **4** and **5**, respectively, and the combined yields of these latter mixed products, which contained a preponderance of the α,β-isomer, were in the range of

[†] I thank Professor G.H. Posner and Grace Davison, St. Neots, Cambs, UK for their help in identifying and tracing this product.

36–49%. The product ratio of the α,α- and α,β-L-isomers is thus at variance with those reported in the related synthesis in the D-series.[5m] Isolation of the α,β-isomer **4** could be achieved by further chromatography of the isomer mixture.

Two faster and close-running components isolated from the reaction mixture could be separated to 90% purity. NMR spectroscopy indicated these to be the α- and β-isomers of benzyl 2,3,4,6-tetra-*O*-benzyl-L-glucopyranoside, (**6**) and (**7**), respectively, which was confirmed by comparison with the corresponding D-isomers, synthesised by benzylation of 2,3,4,6-tetra-*O*-benzyl-D-glucose. Formation of the benzyl glucosides (combined yield, 8.5%) which had not been noted previously,[5m] arises, presumably, through *O*-benzyl cleavage induced by action of trimethylsilyl trifluoromethanesulphonate to form benzyl trifluoromethanesulphonate with concomitant formation of a trimethylsilyl derivative of the carbohydrate moiety, the former then acting as a benzylating agent on the unreacted hemiacetal **2**.

When the α,β-octabenzyl ether **4** was subjected to the synthetic reaction conditions, isomerisation to the α,α-isomer **3** took place to a minor extent and the anomeric benzyl glycosides **6** and **7** were also formed.

Hydrogenation of the α,α-octabenzyl ether **3** over palladium–charcoal occurred slowly over 12 days to afford L-trehalose (**1**) with the expected optical rotation and which was characterised as its octaacetate **8**. Although a melting point for the dihydrate of D-trehalose is regularly reported throughout the chemical literature the difficulties in obtaining a repeatable and sharply defined value for it are not widely recognised despite an important report on the subject in 1962.[9] These difficulties are associated with the different forms of trehalose arising from various degrees of hydration of the disaccharide, a subject of continuing and current interest.[10] Further caution is also necessary in measuring and recording data on the dimorphic octaacetate which in the D-series has a melting point of ~79 °C on recrystallisation from ethanol,[9] elevated to ~102 °C on drying at ~60 °C.[11] An exactly similar behaviour was observed for the enantiomer, octaacetate **8**.

Other procedures (not reported in Section 3) were investigated in the D-series for preferential formation of the α,α-octabenzyl ether. However, whilst all gave the three isomeric disaccharide derivatives none gave any significant advantage, as indicated by TLC. Thus, reaction of 2,3,4,6-tetra-*O*-benzyl-D-glucosyl chloride[12] with the parent hemiacetal in toluene in the presence of silver trifluoromethanesulphonate, reaction of the chloride and hemiacetal in dichloromethane in the presence of *N*,*N*-diisopropylethylamine and tetrabutylammonium chloride,[13] and reaction of trichloroacetyl 2,3,4,6-tetra-*O*-benzyl-D-glucopyranoside with the hemiacetal in dichloromethane in the presence of trimethylsilyl trifluoromethanesulphonate[14] all afforded similar product ratios. In our hands, the previously reported[5g,5h] reaction of 2,3,4,6-tetra-*O*-benzyl-D-glucose with triflic anhydride in dichloromethane gave a slightly more complex reaction mixture than that obtained with trimethylsilyl trifluoromethanesulphonate.‡

L-Trehalose (**1**) was inert to the enzyme α,α-trehalose glucohydrolase under conditions which brought about complete cleavage of D-trehalose to D-glucose.

3. Experimental

3.1. General methods

^1H and ^{13}C NMR spectra were recorded on a JEOL EX270 FT spectrometer, a Varian Gemini 2000 FT spectrometer or a Varian Unity*plus* FT spectrometer in $CDCl_3$ with Me_4Si as internal standard unless stated otherwise; spectra recorded in D_2O had acetone (δ_H 2.22, δ_C 30.89)[15] as internal reference. Resonance allocations were made with the aid of ^1H–^1H COSY and ^1H–^{13}C HSQC experiments when necessary. For compounds **1**, **3**, and **8**, in which the two pyranosyl rings are related by symmetry, NMR data are recorded for one pyranosyl moiety only. Optical rotations were measured at 20 °C with a Perkin–Elmer 141 polarimeter. Mass spectra were recorded by the EPSRC Mass Spectrometry Service at the University College of Swansea. Thin layer chromatography (TLC) was performed on pre-coated plates of silica gel with fluorescent indicator (Machery-Nagel SIL G UV_{254}). Detection was either by viewing under UV light (254 nm), or by spraying with a 10% H_2SO_4, 1.5% molybdic acid, 1% ceric sulfate spray followed by heating to 150 °C. Column chromatography was performed on Kieselgel 60 (70–230 mm mesh, Merck) and preparative layer chromatography (PLC) on Kieselgel 60 PF_{254} containing gypsum (Merck). When mixed solvents were used, the ratios given are v/v. Solvent A is CH_2Cl_2–EtOAc, 98.5:1.5, and solvent B is MeOH–EtOAc, 1:1. Organic solutions were dried over anhydrous Na_2SO_4. Dichloromethane was distilled form calcium hydride. 2,3,4,6-Tetra-*O*-benzyl-L-glucose (**2**), which has been reported only in a patent,[6] was prepared from L-glucose by a procedure similar to that used to synthesise the D-isomer,[7] via the isomeric mixture of α and β allyl L-glucopyransosides, allyl 2,3,4,6-tetra-*O*-benzyl-L-glucopyranosides and 1-propenyl 2,3,4,6-tetra-*O*-benzyl-L-glucopyranosides, except that isomerisation of the *O*-allyl to the

‡ Interestingly, the two reported preparations of the octabenzyl ether of D-trehalose under almost identical conditions by this method gave the α,α- and α,β-isomers in ratios of almost 2:1[5g] and 1:2,[5h] respectively, suggesting subtle effects may control the stereochemical outcome of the reaction.

O-(1-propenyl) group was achieved with potassium *t*-butoxide in a mixture of toluene and Me_2SO;[8] it had mp 149–151 °C, lit.[6] mp 145.5–146.5 °C; $[\alpha]_D$ −24.1° (*c* 0.2, $CHCl_3$), lit.[6] $[\alpha]_D$ −21.1°; for D-isomer[7] mp 153–155 °C; $[\alpha]_D$ +20.9° (*c* 3.5, $CHCl_3$). SYLOSIV®A4, a highly porous, crystalline aluminosilicate with pore openings of approx 4 Å, is a product of W. R. Grace & Co. α,α-Trehalose glucohydrolase (from porcine kidney, EC 3.2.1.28) was obtained as a buffered aqueous glycerol solution from Sigma.

3.2. Preparation of octa-*O*-benzyl-L-trehalose (3) and 2,3,4,6-tetra-*O*-benzyl-α-L-glucopyranosyl 2,3,4,6-tetra-*O*-benzyl-β-L-glucopyranoside (4)

A procedure based on that described by Posner and Bull[5m] for the D-isomer was followed. A solution of 2,3,4,6-tetra-*O*-benzyl-L-glucose (**2**) (1 g, 1.85 mmol) in CH_2Cl_2 (20 mL), containing SYLOSIV® A4 molecular sieve (0.51 g) was stirred at room temperature for 15 min and trimethylsilyl trifluoromethanesulphonate (0.72 mL, 3.7 mmol) was then rapidly added dropwise, causing the development of a pink/red/colouration. After $4^1/_2$ min, TLC (solvent A) showed the disappearance of starting material (R_f 0.06) and the appearance of the 1,1′-linked disaccharides (R_f 0.15, 0.18, and 0.22 for α,β-, β,β-, and α,α-stereoisomers) in addition to two very close-running components **A** and **B** (R_f 0.42 and 0.45). The reaction was quenched by addition of saturated aq $NaHCO_3$ (20 mL), the mixture was filtered through kieselguhr, and the filtrate separated into organic and aqueous components. The aqueous layer was extracted with CH_2Cl_2 (20 mL) and the combined organic layers were washed with aq $NaHCO_3$, water, dried and concentrated. The syrupy material was separated by PLC (solvent A) to yield a mixture of the α,β-, and β,β-1,1′-disaccharides[§] **4** and **5**, respectively (0.35 g with the former in great preponderance), the α,α-disaccharide **3** (0.26 g), and a mixture of the most mobile components **A** and **B** (0.1 g), which was separated (to approx 90% purity of each isomer) by further PLC in the same solvent system and shown to be an approximately equimolar mixture of the isomeric benzyl 2,3,4,6-tetra-*O*-benzyl-L-glucopyranosides, **6** and **7**. Data for the products isolated:

3.2.1. Octa-*O*-benzyl-L-trehalose (3). Syrup, $[\alpha]_D$ −82.7° (*c* 0.3, $CHCl_3$), lit.[5g] for D-isomer $[\alpha]_D$ +83° (*c* 1.6, $CHCl_3$); ^{1}H NMR (300 MHz): δ 3.30 (dd, 1 H, $J_{5,6}$ 1.4, $J_{6,6'}$ 10.4 Hz, H-6), 3.44 (dd, 1 H, $J_{5,6'}$ 2.9 Hz, H-6′), 3.51 (dd, 1 H, $J_{1,2}$ 3.3, $J_{2,3}$ 9.5 Hz, H-2), 3.60 (t, 1 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 3.94 (t, 1 H, H-3), 4.09 (br d, 1 H, H-5), 4.30 and 4.48 (each d and 1 H, $J_{A,B}$ 12.2 Hz, OCH_2Ph), 4.37 and 4.73 (each d and 1 H, $J_{A,B}$ 10.7 Hz, OCH_2Ph), 4.60 (2 H, s, OCH_2Ph), 4.78 and 4.91 (each d and 1 H, $J_{A,B}$ 11 Hz, OCH_2Ph), 5.15 (d, 1 H, H-1), 7.00–7.40 (complex, 20 H, 4× Ph); ^{13}C NMR (75 MHz): δ 68.14 (C-6), 70.61, 72.68, 73.48, 75.06, 75.58, 77.69, 79.39, 81.8, 94.47 (C-1), 127.48, 127.56, 127.75, 127.97, 128.05, 128.06, 128.41, 137.95, 138.33, 138.47, 138.01 (Ar–C), lit.[5g] for D-isomer δ 94.26 (C-1); HRMS: Calcd for $C_{68}H_{74}NO_{11}$ (M + NH_4): 1080.5262; Found: *m*/*z* 1080.5260.

3.2.2. 2,3,4,6-Tetra-*O*-benzyl-α-L-glucopyranosyl 2,3,4,6-tetra-*O*-benzyl-β-L-glucopyranoside (4). Syrup, $[\alpha]_D$ −45.7° (*c* 0.4, $CHCl_3$), lit. [5j] for D-isomer $[\alpha]_D$ +46° (*c* 1.9 $CHCl_3$); ^{1}H NMR (400 MHz): δ 3.40–3.71 (complex, 9 H, H-2a, 6a, 6′a, 2b, 3b, 4b, 5b, 6b, 6′b), 3.74 (t, 1 H, $J_{3a,4a} = J_{4a,5a} = 9.4$ Hz, H-4a), 4.08 (t, 1 H, $J_{2a,3a}$ 9.4 Hz, H-3a), 4.15 (br d, 1 H, H-5a), 4.24–5.12 (complex, 17 H, H-1b, 8× OCH_2Ph), 5.15 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1a), 7.10–7.40 (complex, 40 H, 8× Ph); ^{13}C NMR (75 MHz): δ 68.01, 68.95, 71.82, 73.15, 73.36 (× 3C), 74.52, 74.99 (× 3C), 75.58, 75.77, 77.65, 79.68, 81.75, 82.00, 84.71, 99.54 (C-1a), 104.25 (C-1b), 127.44, 127.64, 127.74, 127.83, 128.02, 128.31, 128.45, 137.17, 138.31, 138.69, 138.92 (Ar–C), lit.[16] for D-isomer δ 99.7 (C-1a) and δ 104.4 (C-1b); HRMS: Calcd for $C_{68}H_{74}NO_{11}$ (M + NH_4): 1080.5262; found: *m*/*z* 1080.5256.

3.2.3. Benzyl 2,3,4,6-tetra-*O*-benzyl-α-L-glucopyranoside (6). Syrup, (solvent A, R_f 0.45), ^{1}H NMR (400 MHz): δ 3.49 (dd, 1 H, $J_{1,2} = 3.6$, $J_{2,3} = 9.6$ Hz, H-2), 3.50 (dd, 1 H, $J_{5,6} = 2$, $J_{6,6'} = 10.4$ Hz, H-6), 3.58 (dd, 1 H, $J_{3,4} =$ 9.2, $J_{4,5} = 10$ Hz, H-4), 3.63 (dd, 1 H, $J_{5,6'} = 3.2$, Hz, H-6′), 3.73 (ddd, 1 H, H-5), 3.97 (br t, 1 H, H-3), 4.36–4.95 (complex, 10 H, 5× OCH_2Ph), 4.78 (d, 1 H, H-1), 7.00–7.40 (complex, 25 H, 5× Ph); ^{13}C NMR (75 MHz): δ 68.40 (C-6), 69.11 (*C*H_2), 70.36 (C-5), 72.99 (*C*H_2), 73.45 (*C*H_2), 75.06 (*C*H_2), 75.70 (*C*H_2), 77.72 (C-4), 79.91 (C-2), 82.15 (C-3), 95.65 (C-1), 127.74, 127.81, 128.01, 128.43, 137.29, 138.06, 138.28, 138.38 138.97 (Ar–C); HRMS (for mixed α- and β-isomers): Calcd for $C_{41}H_{46}NO_6$ (M + NH_4): 648.3325; Found: *m*/*z* 648.3323.

3.2.4. Benzyl 2,3,4,6-tetra-*O*-benzyl-β-L-glucopyranoside (7). Syrup, (solvent A, R_f 0.42), ^{1}H NMR (400 MHz): δ 3.40 (ddd, 1 H, $J_{4,5} = 9.2$, $J_{5,6} = 4.8$, $J_{5,6'} = 2$ Hz, H-5), 3.46(dd, 1 H, $J_{1,2} = 7.6$, $J_{2,3} = 8.8$ Hz, H-2), 3.55 (complex, 1 H, $J_{3,4} = 9.2$, $J_{4,5} = 9.2$ Hz, H-4), 3.58 (complex, 1 H, H-3), 3.63 (dd, 1 H, $J_{6,6'} = 10.8$ Hz, H-6), 3.69 (dd, 1 H, H-6′), 4.44 (d, 1 H, H-1), 4.42–4.93 (complex, 10 H, 5× OCH_2Ph), (7.00–7.40 (complex, 25 H, 5× Ph); ^{13}C NMR (75 MHz): δ 68.93 (C-6), 71.12 (*C*H_2), 73.47 (*C*H_2), 74.86 (*C*H_2), 74.90 (C-5), 74.96 (*C*H_2), 75.67

[§] This mixture was subjected to column chromatography on a pre-packed silica-gel column with CH_2Cl_2 as eluent to afford the α,β-isomer.

(CH_2), 77.89 (C-4), 82.31 (C-2), 84.74 (C-3), 102.64 (C-1), 127.67, 127.82, 127.94, 128.02, 128.24, 128.43,137.58, 138.22, 138.29, 138.50 138.69 (Ar–C).

3.3. Benzyl 2,3,4,6-tetra-*O*-benzyl-α and β-D-glucopyranosides

Benzylation of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranoside with benzyl bromide/sodium hydride in 1,2-dimethoxyethane afforded a mixture of the α- and β-benzyl glycosides, indistinguishable by TLC, and ^1H and ^{13}C NMR spectroscopy from the mixture of corresponding L-isomers isolated as by-products in the synthesis of octa-*O*-benzyl-L-trehalose.

3.3.1. L-Trehalose (1). A solution of octa-*O*-benzyl-L-trehalose (**3**, 0.25 g, 0.235 mmol) in EtOAc–EtOH (1:9 v/v, 20 mL) containing HOAc (0.1 mL) was stirred under an atmosphere of hydrogen in the presence of 10% palladium–charcoal catalyst (75 mg) for 12 days when TLC (solvent B) showed reaction was complete. After filtration, through kieselguhr and then Celite, the filtrate was concentrated to afford solid which was stored at approx 18 °C in a vacuum desiccator over P_2O_5 and KOH to give as the solid dihydrate the title compound **1** (0.077 g; 87%); $[\alpha]_D$ −184.5° (*c* 0.25, H_2O), lit.[9] for dihydrate of D-isomer $[\alpha]_D$ +180° (*c* 1, H_2O), lit.[9] for anhydrous D-isomer $[\alpha]_D$ +206° (*c* 1.3, H_2O); ^1H NMR (300 MHz, D_2O,): δ 3.45 (t, 1 H, $J_{3,4} = J_{4,5} = 9.3$ Hz, H-4), 3.65 (dd, 1 H, $J_{1,2} = 3.6$, $J_{2,3} = 9.9$ Hz, H-2), 3.70-3.90 (complex, 4 H, H-3,5,6,6′), 5.20 (d, 1 H, H-1); ^{13}C NMR (75 MHz, D_2O): δ 61.22 (C-6), 70.41, 71.75, 72.86, 73.23, 93.98 (C-1)¶; HRMS: Calcd for $C_{12}H_{26}NO_{11}$ ($M + NH_4$): 360.1506; Found: *m*/*z* 360.1506.

3.4. Octa-*O*-acetyl-L-trehalose (8)

Acetylation of L-trehalose with acetic anhydride–pyridine afforded a syrup which was crystallised from EtOH to give the title compound **8**, mp 78–80 °C, and mp 101–101.5 °C after drying at 55 °C at atmospheric pressure for 12 h; $[\alpha]_D$ −162° (*c* 0.74, $CHCl_3$); {for D-isomer: lit.[11a] mp 70–75 °C, and mp 100–102 °C after drying at 61 °C/12 mm; lit. [11b] mp 68–75 °C, mp 102–103 °C after drying at 60 °C/12 mm, $[\alpha]_D$ +161.9° (*c* 1.0, $CHCl_3$); lit.[9] mp 78–79 °C and report of dimorphism}; ^1H NMR (400 MHz): δ 1.97, 1.99, 2.01, 2.02 (each s and 3H, 4 × CH_3CO), 3.94 (dd, 1 H, $J_{5,6} = 2$, $J_{6,6'} = 12.1$ Hz, H-6), 3.98 (ddd, $J_{4,5} = 10.3$, $J_{5,6'} = 5.7$ Hz, H-5), 4.18 (dd, 1 H, H-6′), 4.97 (dd, 1 H, $J_{1,2} = 3.8$, $J_{2,3,} = 9.3$ Hz, H-2), 4.98 (t, 1 H, $J_{3,4}$ 9.4 Hz, H-4), 5.22 (d, 1 H, H-1), 5.43 (t 1 H, H-3); ^{13}C NMR (100 MHz): δ 20.79, 20.82 (× 2C) and 20.90 (4 × CH_3CO), 61.92 (C-6), 68.37, 68.70, 70.03, 70.57, 92.44 (C-1), 169.79, 169.83, 170.18 and 170.83 (4 × $CH_3C{=}O$); HRMS: Calcd for $C_{28}H_{42}NO_{19}$ ($M + NH_4$): 696.2351; Found: *m*/*z* 696.2349.

¶ These NMR data are in good agreement with those reported[17] for D-trehalose.

3.5. Isomerisation of 2,3,4,6-tetra-*O*-benzyl-α-L-glucopyranosyl 2,3,4,6-tetra-*O*-benzyl-β-L-glucopyranoside (4)

A solution of **4** (50 mg) in dry CH_2Cl_2 (1 mL) was stirred with SYLOSIV® A4 molecular sieves at room temperature and trimethylsilyl trifluoromethanesulphonate (0.036 mL) was added. The reaction was quenched after 5 min by addition of aq $NaHCO_3$. Examination of the organic layer by TLC (solvent A) showed the presence of the octa-*O*-benzyl-L-trehalose (**3**) in addition to **4** as well as the benzyl 2,3,4,6-tetra-*O*-benzyl-α and β-L-glucopyranosides **6** and **7**.

3.6. Action of trehalase (α,α-trehalose glucohydrolase, EC 3.2.1.28) on D- and L-trehalose

To aqueous solutions of D- and L-trehalose (6 mg in 2 mL) was added a solution of the trehalase (10 mL of 0.2 mL solution in glycerol containing 1 unit of the enzyme) and the solutions were stored at 20 °C for 30 h. TLC (solvent B) then indicated complete hydrolysis of the D-isomer to D-glucose (R_f 0.76) whereas the L-isomer (R_f 0.51) was unchanged.

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