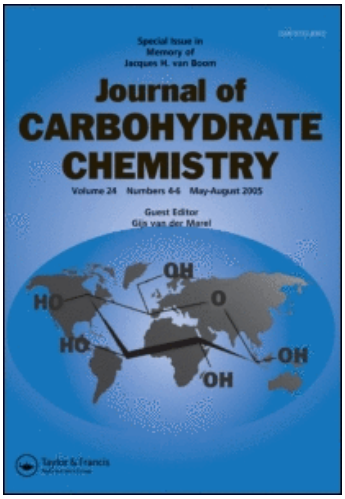

Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

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To cite this Article Baddeley, Thomas C. and Wardell, James L.(2009) 'Synthesis of Per- and Poly-Substituted Trehalose Derivatives: Studies of Properties Relevant to Their Use as Excipients for Controlled Drug Release', Journal of Carbohydrate Chemistry, 28: 4, 198 — 221

To link to this Article: DOI: 10.1080/07328300902887672

URL: http://dx.doi.org/10.1080/07328300902887672

Journal of Carbohydrate Chemistry, 28:198–221, 2009

ISSN: 0732-8303 print / 1532-2327 online
DOI: 10.1080/07328300902887672

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Synthesis of Per- and Poly-Substituted Trehalose Derivatives: Studies of Properties Relevant to Their Use as Excipients for Controlled Drug Release

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Per- and poly-substituted oligosaccharide derivatives, with trehalose cores, have been prepared and assessed for their potential for use as excipients in controlled-release formulations. The synthesized compounds, generally with acyl and amido substituents, included 6,6′-*N*,*N*′-diamido-6,6′-dideoxy-α,α-trehalose derivatives, 6,6′-bis(1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronyl)-α, α-trehalose derivatives, 2,2′,3,3′-tetra-*O*-acetyl-6,6′-*bis*-(1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronyl)-4,4′-di-*O*-acyl-α,α-trehalose, 2, 2′, 3, 3′-tetra-*O*-acetyl-6-(1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronyl)-4,4′,6′-tri-*O*-acyl-α,α-trehalose, and 2,2′,3,3′,4,4′-hexa-*O*-acetyl-6,6′-*bis*-(1,2,3,4-tetra-*O*-acetyl-6-*O*-succinyl-β-D-glucopyranuronyl)-α,α-trehalose. Compounds were characterized by NMR, IR, MS and optical rotations; elemental analyses; or HRMS. The compounds formed amorphous materials either on fast quenching of melts or on spray drying. Properties, used in the initial assessment of the potential as controlled-release excipients, were $\log_{10}$ P and glass transition, T_g, values.

Keywords Trehalose derivatives; Glass transition temperatures; Controlled release

Received July 19, 2008; accepted March 11, 2009.
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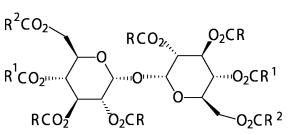
INTRODUCTION

The controlled release of actives, such as drugs, is of great interest in modern therapeutic drug delivery, due to benefits arising from single-dose treatments, increased pharmacologic efficacy, extended periods of action, greater flexibility in administration, and improved targeting of specific sites.[1] Among the materials considered for use in controlled-release systems are polymers, both man-made and natural systems,[2–9] glasses,[10,11] and materials based on carbohydrates (e.g., cyclodextrin).[12–15] Other carbohydrate-based systems include trehalose-hydroxyethylcellulose microspheres[16] and derivatives of di- and other oligomeric saccharides, especially trehalose derivatives.[17,18] Many carbohydrates form reasonably stable amorphous phases, including glass phases, by rapid cooling from melts. Such phases are suitable for use as matrices for hosting, stabilization, and release of bioactive materials.[19,20] The melt temperatures of these derivatives are sufficiently low to safely incorporate different bioactives. In addition, spray drying techniques can also be employed to generate encapsulated materials from solutions.

We have prepared a number of di-, tri-, and tetra-saccharide derivatives, with trehalose cores, and have investigated selected properties that we consider relevant to their use as excipients. For successful use in controlled-release systems in aqueous media, an ideal excipient is considered to have a suitable partition between water and lipids, have stability in aqueous media, and form an amorphous phase having a glass transition temperature, T_g, above ambient and body temperatures. Too rapid dissolution in aqueous media and interactions with water leading to plasticization and devitrification will all limit the utility. Too low a glass transition temperature would allow undesirable devitrification of the host excipient to occur, usually leading to more rapid release of its guests. By suitable derivatization, oligosaccharides can be obtained having suitable T_g and $\log_{10}$ P values. The $\log_{10}$ P value, the ability to partition between water and octanol, is a useful measure of the balance between the hydrophilicity and hydrophobicity of a material: values between 0 and 4 are taken as being indesirable.

Important considerations for complex saccharide excipients are their synthesis and purification, especially with regard to the problem of mixed anomer products. The choice of trehalose derivatives was made due to the symmetry of the parent disaccharide, α,α-trehalose; the nonreducing properties of the trehalose system; and the results of a published study.[17] The latter study reported the viable use of per-esterified trehalose derivatives (see Fig. 1) in the form of microparticles for the delivery of insulin via inhalation. We wish to report the preparation and study of properties of other per- and poly-substituted trehalose derivatives in order to establish a larger number of potentially useful compounds for use in drug delivery systems generally, and not exclusively for delivery via inhalation.



R = R^1 = R^2 = Me
R = R^1 = Me; R^2 = Pr^i
R = R^1 = Me; R^2 = *cyclo*-C_6H_{11}
R = R^1 = Me; R^2CO_2 = 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronyl
R = Pr^i ; R^1 = Me; R^2CO_2 = 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronyl

Figure 1: Trehalose derivatives used by Davidson et al[17].

We now wish to report our results.

RESULTS AND DISCUSSION

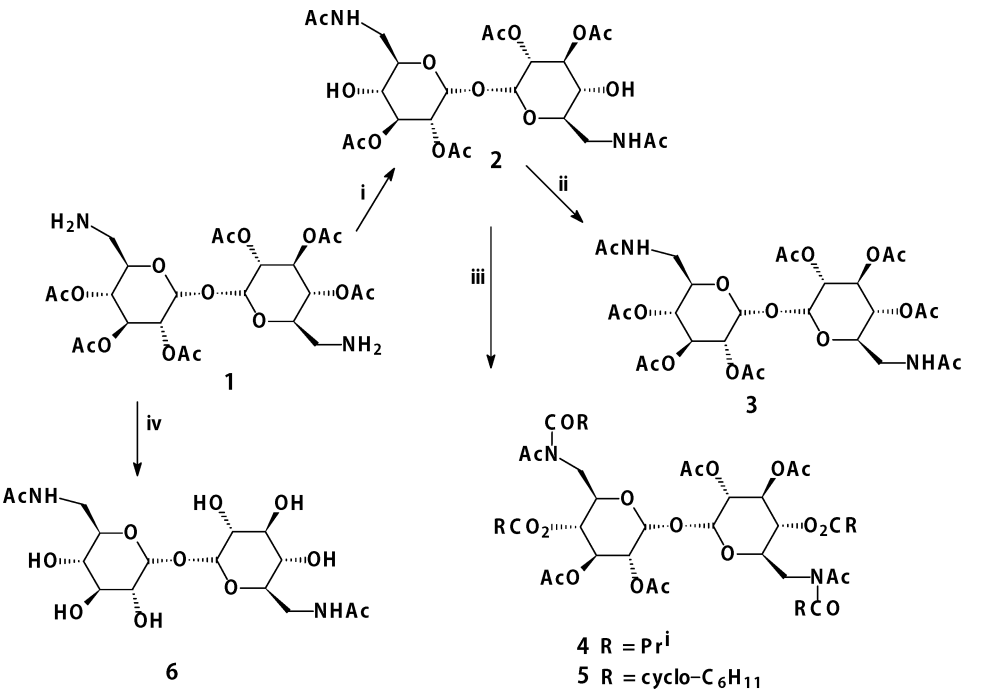
Synthesis

The reported study[17] involved octa-acylated trehalose derivatives including two hexa-O-acyl-(1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronyl)-*α*,*α*-trehalose derivatives (Fig. 1). In our study, we have extended the types of derivatives studied to more carbon-rich compounds and to poly-substituted compounds, with free hydroxyl groups, rather than exclusively to per-substituted compounds. Compounds studied include 6,6′-*N*,*N*′-diamido-6,6′-dideoxy-*α*,*α*-trehalose derivatives, 6,6′-bis(1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronyl)-*α*,*α*-trehalose derivatives, and pseudo-tetrasaccaharides such as 2,2′,3,3′,4,4′-hexa-*O*-acetyl-6,6′-*bis*-(1,2,3,4-tetra-*O*-acetyl-6-*O*-succinyl-*β*- D-glucopyranuronyl)-*α*,*α*-trehalose.

Formation of 6,6′-N,N′-diamido-6,6′-dideoxy-α,α-trehalose derivatives

Amido-trehalose derivatives, as a group, had not been targeted previously as potential drug excipients. 2,2′,3,3′,4,4′-Hexa-*O*-acetyl-6,6′-diamino-6,6′-dideoxy-*α*,*α*-trehalose, **1**, had been prepared by us[21] by the reduction of 2,2′,3,3′,4,4′-hexa-*O*-acetyl-6,6′-di-azido-6,6′-dideoxy-*α*,*α*-trehalose, using triphenylphosphine in wet reagent-grade THF, in a Staudinger-type reaction.[22–25] The use of PPh_3 in THF gave good yields of **1**, in contrast to the literature reports of either poor yields or its in situ conversion to 6,6′-di-*N*-acetyl-2,2′,3,3′-tetra-*O*-acetyl-6,6′-dideoxy-*α*,*α*-trehalose, **2**, on using hydrogen and Pd/C.[22,26] Direct precipitation of **1** from the THF solution facilitated its separation and **1** simply purified by washing with 1,2-dichloroethane: yields greater than 90% were obtained when reaction times greater than 4 days were employed.

Rearrangement of **1** to **2** was most readily achieved in pyridine solution, although aqueous or methanolic solutions could also be used (see Sch. 1). The acetyl migration could be followed using the acetamido methyl protons in the ^{1}H NMR spectrum: of interest, additional peaks for an unsymmetric intermediate species could be detected during the reaction. Even after long reaction times, the solution ^{1}H NMR spectrum indicated sets of peaks for both **1** and



Scheme 1. Reagents: (i) py, 3 h, rt; (ii) Ac_2O, py; (iii) Pr^iCOCl or cyclo-$C_6H_{11}COCl$, py, 4 h; (iv) NaOMe, HOMe.

2, but on work-up, only product **2**, showing complete migration, was isolated. Thus, in solution the reaction apparently only proceeds to an equilibrium position, but that work-up drives it to completion.[27] Of interest, the conditions for the acetyl migration in **1** to **2** (a migration from *O* to *N*) occurs under much milder conditions than required for the more frequently observed *O* to *O* acetyl migrations. For example, under the same conditions used to transform **1** to **2**, no acetyl migration occurred with 2,2′, 3,3′,4,4′-hexa-*O*-acetyl-α, α-trehalose.

Treatment of **2** with acetic anhydride in pyridine provided **3**, an octa-acylated compound. In contrast, the more reactive acylating agents, isobutyroyl and cyclohexanoyl chlorides, furnished the per-(deca-)acylated derivatives, **4** and **5**. Compound **3** could also be obtained directly from **1** on reaction with excess acetic anhydride. Attempts to couple **1** with 1,2,3,4-tetra-*O*-acetyl-β-D-glucuronic acid,[28] in the presence of 1,3-dicyclohexylcarbodiimide (DCC), or the polymer-supported coupling agent, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide on Merrifield resin, failed to provide 4,4′-hexa-*O*-acetyl-6,6′-bis(1,2,3,4-tetra-*O*-acetyl-β-D-glucuronamido)-1,1′-dideoxy-α,α-trehalose. Instead **2** was isolated (i.e., acetyl migration had resulted): this further indicated that the free hydroxyls at the 4,4′ sites in **2** are insufficiently reactive to couple with 1,2,3,4-tetra-*O*-acetyl-β-D-glucuronic acid.

We found a slightly higher m.p. and larger $[\alpha]_D$ value for **3** than that reported [22]; this we assume is due to us having a purer product.

Acetyl migration in **1** can also be brought about under basic conditions. Treatment of **1** with sodium methoxide in methanol[29] led to formation of 6,6′-di-*N*-acetyl-6,6′-dideoxy-*α*,*α*-trehalose, **6** (i.e., both acetyl migration from the 4 to 6 sites and deacetylation at the remaining positions had occurred).

Preparations of 6,6′-bis(1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronyl)-α, α-trehalose derivatives

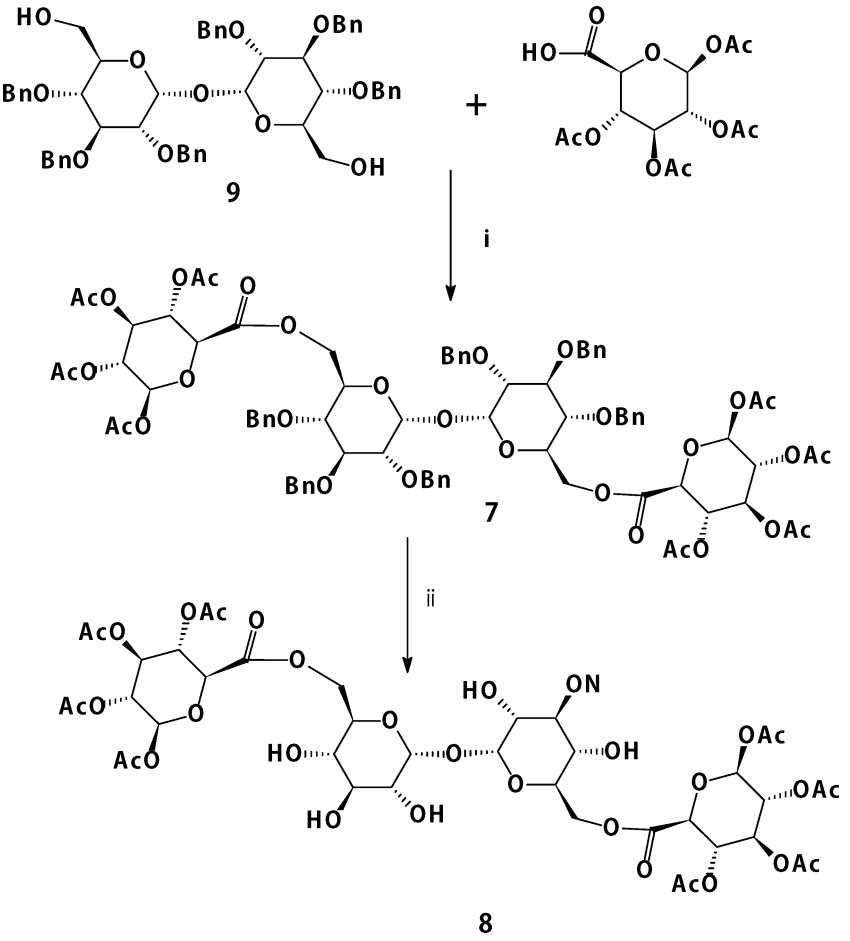
6,6′-*Bis*-(1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronyl)-2,2′,3,3′,4,4′-hexa-*O*-benzyl-*α*,*α*-trehalose, **7**, and 6,6′-*bis*-(1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronyl)- *α*,*α*-trehalose, **8**, were prepared, using as the starting compound the known 2,2′,3,3′,4,4′-hexa-*O*-benzyl-*α*,*α*-trehalose, **9**,[22,30] (see Sch. 2).

Reaction of **9** with 1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronic acid, in the presence of *p*-(dimethylamino)pyridine (DMAP) and DCC, in acetonitrile produced 6,6-*bis*-(1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronyl)-2,2′,3,3′,4,4′-hexa-*O*-benzyl-*α*,*α*-trehalose, **7**, albeit in a low yield after extensive purification. Debenzylation of **7** by H_2/Pd/C gave 6,6′-b*is*-(1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronyl)-*α*,*α*-trehalose, **8**.

The compounds, 2,2′,3,3′-tetra-*O*-acetyl-6,6′-*bis*-(1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronyl)-*α*,*α*-trehalose, **10;** 2,2′,3,3′-tetra-*O*-acetyl-6,6′-*bis*-(1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronyl)-4,4′-di-*O*-isobutyryl-*α*,*α*-trehalose, **11**; and 2,2′,3,3′-tetra-*O*-acetyl-6,6′-*bis*-(1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronyl)-4,4′-di-*O*-cyclohexanecarbonyl-*α*,*α*-trehalose, **12**, were obtained as shown in Scheme 3.

2,2′,3,3′-Tetra-*O*-acetyl-*α*,*α*-trehalose, **13**, was obtained from trehalose via the following sequence: (i) benzylidation, using $PhCH(OMe)_2$/p-toluenesulfonyl chloride/DMF, (ii) acetylation, using acetyl anhydride/pyridine, and (iii) debenzylidation, using acetic acid at 45°C. Reaction of **13** with 1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronic acid (1:2 mole ratio), in the presence of DCC (two equivalents) and DMP in MeCN, gave a mixture of **10** and the product of a mono-reaction, 2,2′,3,3′-tetra-*O*-acetyl-1-(1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronyl)-*α*,*α*-trehalose, **14**. As expected, reactions with **13** only occurred at the less hindered primary alcohol groups. We subsequently established that DCC is partially consumed, even in the presence of DMAP, as an adduct with 1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronic acid. Separation of **10** and **14** could be readily made using column chromatography.

Reaction of the separated compounds with acyl chlorides led to the expected products. However, it was more convenient and efficient to treat the unseparated mixture of **10** and **14** with acyl chlorides and then to separate products using column chromatography. Thus, reaction with isobutyryl chloride, **10** and **14,** gave a readily separable mixture of

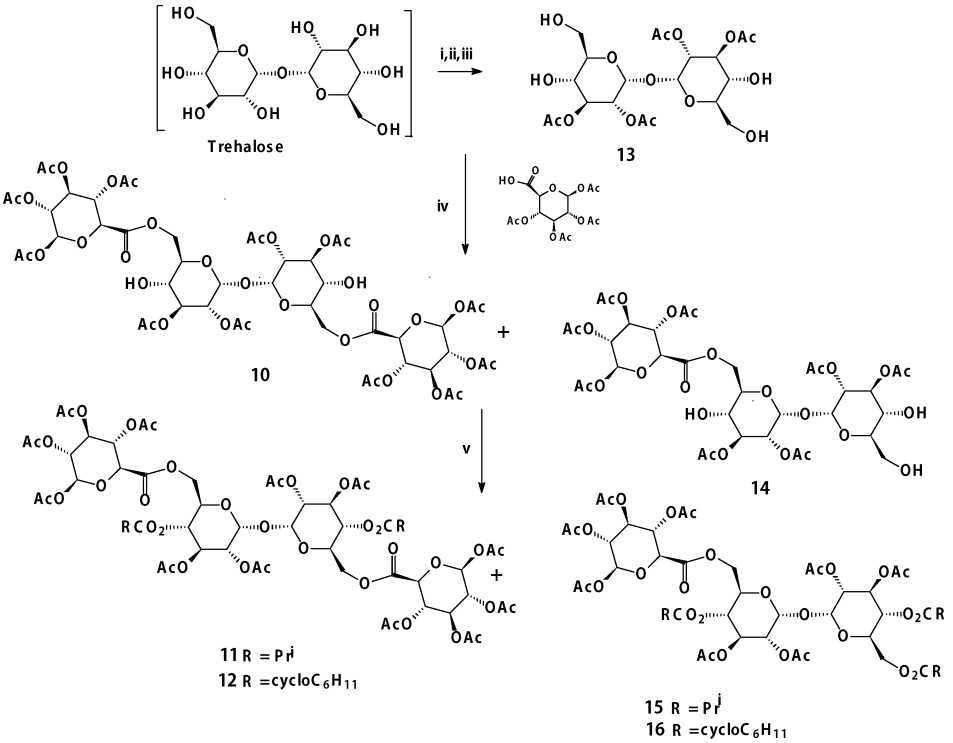


Scheme 2. Reagents: (i) DCC, DMAP, MeCN, 4 h; (ii) H_2, Pd/C, MeOH, 3 d.

2,2′,3,3′-tetra-*O*-acetyl-6,6′-*bis*-(1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronyl)-4,4′-di-*O*-isobutyryl-*α,α*-trehalose, **11**, and 2,2′,3,3′-tetra-*O*-acetyl-1-(1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronyl)-4,4′,1′-tri-*O*-isobutyryl-*α,α*-trehalose, **15**. Similarly, **10** and **14** produced with cyclohexanoyl chloride a readily separated mixture of 2,2′,3,3′-tetra-*O*-acetyl-6,6′-*bis*-(1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronyl)-4,4′-di-*O*-cyclohexanecarbonyl-*α,α*-trehalose, **12**, and 2,2′,3,3′-tetra-*O*-acetyl-1-(1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronyl)-4,4′,1′-tri-*O*-cyclohexanecarbonyl-*α,α*-trehalose, **16**.

Preparation of 2,2′,3,3′,4,4′-hexa-O-acetyl-6,6′-bis-(1,2,3,4-tetra-O-acetyl-6-O-succinyl-β-D-glucopyranuronyl)-α,α-trehalose

The succinic acid ester, **17**, a pseudo-tetrasaccharide, derived from two different carbohydrate residues was obtained by an initial reaction of



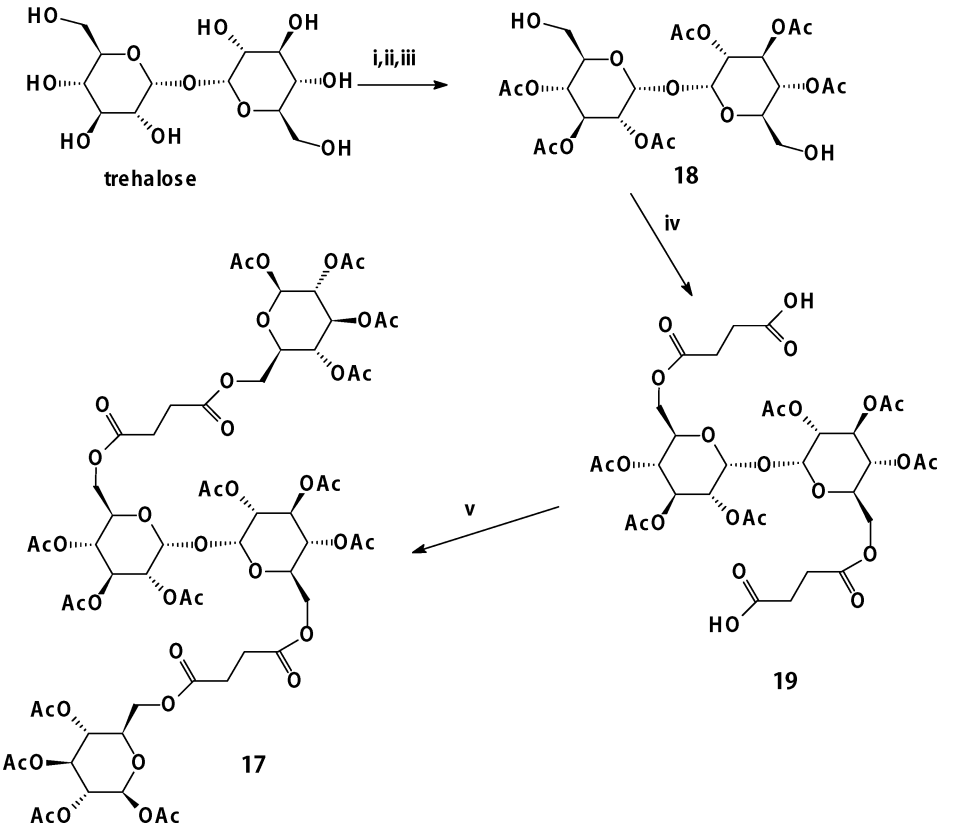
Scheme 3. Reagents: (i) $PhCH(OMe)_2$, p-TsCl, DMF, 50°C, 40 min; (ii) Ac_2O, py, rt, 8 h; (iii) AcOH, 45°C, H_2O, 20 min; (iv) DCC, DMAP, MeCN, RT, 8 h; (v) RCOCl (R = Pr^i or cyclohexyl), py, rt, 8 h.

succinic anhydride with 2,2′,3,3′,4,4′-hexa-*O*-acetyl -α,α-trehalose, **18**, to give **19**, which was then coupled with 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose (see Sch. 4).

Our prepared sample of **18** had a considerably larger α_D value (253°) than that quoted in the literature[36] (158.8°): as our m.p. was higher and sharper (102–103°C vs. 93–96°C), we assume our sample was the purer. However, the m.p. of the intermediate, 6,6′-di-*O*-trityl-α,α-trehalose, used to obtain **18**, was much lower than that quoted in the literature[26] (191–193°C compared to 278–281°C). NMR spectra were as expected for the compound, but a small amount of water was clearly present.

Screening of Compounds Based on T_g and log P_{ow} Value for Potential Use as Excipients

Glass transition temperatures, T_g, and partition coefficients, $\log_{10}$ P values, were determined for the oligosaccharide derivatives (see Table 1). Melting points, T_g values, and remelts of investigated compounds were obtained using heat, cool, and reheat programs in DSC experiments. Melting points are



Scheme 4. Reagents: (i) Ph_3CCl, Ac_2O, py, 2 h; (ii) IR 70 resin, H_2O, MeCN, 5°C, 20 h; (iii) succinic anhydride, py, 8 h; (iv) DMAP, MeCN, rt, 8 h.

quoted as a single temperature as they were taken as the maximum of the melting peak in the DSC trace. Octanol-water partition coefficients (log P_{ow}) were determined using an HPLC method.[31] Being a partition coefficient, $\log_{10}$ P, which is defined as the partition coefficient between 1-octanol and water, is not an absolute measure of hydrophobicity or solubility in water. However, it is assumed that a high value of $\log_{10}$ P indicates a hydrophobic substance having a low solubility in water.

A useful excipient is considered to have a T_g value at least 20°C above temperature of its use and a log P_{ow} value between 0 and 4 for release into aqueous media. Above T_g, devitrification will occur, one consequence of which is the creation of pores or channels, which would allow incipients to be released at increasing rates as crystallization proceeds.

On the basis of the stated criteria for a potentially useful excipient, compounds **10**, **11**, **12**, **14**, **15**, and**17** are worthy of further study. However, compounds **7** (too low a T_g value), **3** (too low a $\log_{10}$ P value), and **5** and **16** ($\log_{10}$ P

Table 1: Properties of oligosaccharide derivatives

Compound	m.p.°C	T_g°C	$Log_{10}P$	Mol. Weight
3	110	72	–1.19	676
4	183	118	4.26	872
5	125	105	8.36	1032
7	53	26	[a]	1570
8	124	61	1.30	1030
10	220	111	2.26	1198
11	96	85	3.30	1338
12	91	88	3.88	1418
14	205	129	1.06	854
15	106	99	3.50	1062
16		[b]	4.93	1182
17	113	95	3.04	1456
octa-O-acetyl-α, α-trehalose	91	51	0.97	592

[a]Not determined as T_g is too low.
[b]Not determined as $log_{10}P$ is too low.

too high) can be immediately eliminated as potentially useful excipients, while compound **4** is marginal.

It is, however, important to keep in mind that any compound incorporated into the excipient could lead to a reduction of the T_g value of the excipient. This was realized with compound **3**, formulated by spray drying with (2S,3S)-(+)-cis-3-acetoxy-5-(2-dimethylaminoethanol)-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one hydrochloride (Ditiazem HCl) or pregna-1,4-diene-3,20-dione-6,7,21-trihydroxy-prednisolone (prednisolone) at 10% loadings. In both cases, there were reductions in the T_g value of pure **3** by 15°C. However, the same drugs incorporated into **4** produced much lower reductions (<5°C).

As well as T_g and $log_{10}P$ values, other factors must be evaluated. For example, the effects of humidity and water on excipients/formulations, which could lead to crystallization and other unwanted results, must be considered: such effects are not necessarily related to $log_{10}P$ values. These and other aspects will be discussed in future articles.

The glass transition and melting point data, listed in Table 1, clearly illustrate that melting points and glass transition temperatures do not correlate with molecular weights even for related per-esterified saccharides, which are free of such strong intermolecular associations as H-O—H hydrogen bonds. Where strong H-bonding can occur as in compounds with free hydroxyl groups, significant differences in $T_g$values are expected. This is exemplified by the T_g/m.p. values for trehalose, 6,6′-yghdiamino-6,6′-dideoxy-α,α-trehalose, and 6,6′-diacetamido-6,6′-dideoxy-α,α-trehalose of 70/202°C, 10/222°C, and 22/84°C, respectively.[31]

Conclusion

While further study is necessary, the T_g and $\log_{10}P$ data for compounds **10**, **11**, **12**, **14**, **15**, and **17** indicate their potential as excipients. These compounds, having a range of T_g and $\log_{10}P$ values within the recommended limits, can further extend the utility of trehalose and trehalose derivatives, such as trehalose octaacetate, already found to be useful in drug delivery systems.

EXPERIMENTAL

General

TLC was carried out on silica using 10% sulphuric acid, iodine, or ninhydrin to develop spots. Melting points were obtained on a Kofler hotstage or a Perkin-Elmer DSC7 Differential Scanning Calorimeter and are uncorrected. IR spectra were recorded in KBr discs using an Atti Mattson FTIR instrument; UV-visible spectra on a Cary 300 UV spectrometer; ^1H and ^{13}C NMR spectra on 250, 300, or 500 MHz Bruker instruments; or mass spectra on a Finnigan Navigator Mass Spectrometer using ES^+ ionization (low resolution); or by the EPSRC mass spectrometer service at the University of Swansea (high resolution) and optical rotations ($deg.cm^2.g^{-1}$ and concentration, c, in $g.100\ mL^{-1}$) on a Bellingham + Stanley Ltd P20 polarimeter. C, H, and N analyses were performed on a Perkin–Elmer 2400 apparatus. A Hewlett Packard HP 1100 instrument was used for HPLC measurements.

Materials characterization

Thermal analysis was carried out on 10-mg samples on a Perkin-Elmer DSC7 Differential Scanning Calorimeter, using heat, cool, and reheat cycles with temperature gradients of 10°C per minute. Heating was taken to at least 20°C above melting point, T_m, and cooling to at least 20°C below the glass transition temperature, T_g. Thermogravimetric analysis was carried on a Perkin Elmer TGA 7 instrument: Pyris 1 software was used.

Octanol-water partition coefficients (log P_{ow}) were determined using an HPLC method.[32] Values of log P_{ow} for test compounds were calculated from the linear correlation of log P_{ow} and capacity factor, K, for the reference materials (benzyl alcohol, methyl benzoate, biphenyl, and bibenzyl), where the capacity factor, K, is defined as $K = (rt_x - rt_o)/rt_o$, where rt_x is the retention time for the compound, and rt_o is the retention time for acetonitrile, under a given set of conditions. The retention times of the new saccharides and reference compounds of known log P_{ow} were measured on a Phenomenex Jupiter (C18 250 mm × 4.6 mm, 5 μm) column with 80:20 acetonitrile:water as the mobile phase, with UV detection at 209 nm.

Syntheses of Compounds

6,6′-Bis-(N-acetylamino)-2,2′,3,3′,4,4′-hexa-O-acetyl-6,6′-dideoxy-α,α-trehalose, **3**

To a solution of 2′,3,3′,4,4′-hexa-*O*-acetyl-6,6′-diamino-6,6′-dideoxy-α,α-trehalose, **1**[9] (2.0 g, 3.4 mmol), in methanol (30 mL) and pyridine (5 mL) was added excess acetic anhydride (2 mL, 14 mmol). After leaving overnight, the solvent was removed under reduced pressure to leave an oil. Diethyl ether was added and the resulting sticky solid was recrystallized from CH_2Cl_2/Et_2O at –22°C; yield 1.5 g (65.7%); m.p. 109–110°C, lit. m.p. (ethyl acetate/ hexane) 92–98°C[22]; $[\alpha]_D^{25}$ (c = 4, $CHCl_3$) 170.4; lit [22] $[\alpha]_D$ ($CHCl_3$) 155.

^{1}H NMR (400 MHz, $CDCl_3$): δ: 1.94 (s, 3H, Me), 1.97 (s, 3H, Me), 2.02 (s, 3H, Me), 2.03 (s, 3H, Me), 3.18 (m, 1H, H-6′), 3.51 (m, 1H, H-5), 3.76 (m, 1H, H-6), 4.83 (t, 1H, J = 9.9 Hz, H-4), 4.85 (dd, 1H, J = 4.0, 9.9, H-2), 5.27 (d, 1H, J = 4.0 Hz, H-1), 5.42 (t, 1H, J = 9.9 Hz, H-3), 5.84 (t, 1H, J = 6.0, N*H*$COCH_3$).

^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.5, 20.6, 20.6, 23.0, 38.9, 69.0, 69.3, 69.5, 70.2, 91.4, 169.8, 169.9, 170.2, 170.3.

IR (KBr, cm^{-1}): *v*: 3554–3411, 1753, 1655, 1543, 1372, 1225, 1040.

MS (FAB): 168.0 (100%), 677.0 (47%, M + H), 227.9 (28%), 698.9 (24%, M + Na).

MS (ES^+, Acc. Mass, M + H): calculated for $C_{28}H_{40}N_2O_{17}$: 677.2405, obtained: 677.2402.

6,6′-Bis-(N-acetylamino)-2,2′,3,3′-tetra-O-acetyl-6,6′-dideoxy-α,α-trehalose, **2**

Diamine, **1** (0.5 g, 0.9 mmol), was dissolved either in pyridine (10 mL), methanol (10 mL), or water (10 mL). The reaction mixtures were left overnight. The aqueous or methanolic solution reaction mixtures were rotary evaporated to leave a white solid, whereas ether was added to the pyridine reaction mixture to precipitate a solid, m.p. 204°C; $[\alpha]_D^{25}$ (c = 3, MeOH) 123.2; lit[22] m.p. (from ethanol/ether) 197–200°C; lit.[22] $[\alpha]_D$ (methanol/water, 3:1) 130.

^{1}H NMR (250 MHz, $CDCl_3$): δ: 1.80 (s, 3H, Me), 1.98 (s, 3H, Me), 2.02 (s, 3H, Me), 2.91 (m, 1H, H-6), 3.38 (t, 1H, J = 9.2 Hz, H-4), 3.54 (m, 1H, H-5), 3.70 (m, 1H, H-6′), 4.79 (dd, 1H, J = 3.7 & 10.37 Hz, H-2), 5.18 (m, 2H, H-1 and H-3), 7.81 (t, J = 4.3 Hz, N*H*$COCH_3$).

^{13}C NMR (63 MHz, $CDCl_3$): δ: 20.3, 20.8, 22.4, 69.0, 69.8, 71.4, 71.7, 90.6, 169.7, 169.9.

IR (KBr): *v*: 3615–3096, 2867, 1739, 1643, 1571, 1437, 1378, 1236, 1141, 1047, 1017.

MS (ES^+): 615.3 (100%, M + Na), 573.3 (6%, M-Ac + Na), 1207.7 (2%, 2M + Na), 531.3 (1%, M-2Ac + Na).

Anal. Calcd for $C_{24}H_{36}N_2O_{15}$: C, 48.65; H, 6.12; N, 4.72. Found: C, 49.01; H, 6.31; N, 4.89.

2,2′,3,3′-Tetra-O-acetyl-6,6′-bis-(N-acetyl-N-isobutyroylamino)-6,6′-dideoxy-4,4′-di-O-isobutyryl-α,α-trehalose, ***4***

To a solution of **3** (2.0 g, 3.4 mmol) in pyridine (20 mL) was added isobutyroyl chloride (3 mL, 10.3 mmol). After stirring overnight, the solvent was removed at reduced pressure to leave an oil, which was poured into water (500 mL). The resulting white precipitate was collected, dried, and recrystallized from ethanol. Further recrystallization was achieved from aqueous methanol. Yield of **4** was 0.9 g (30%); m.p. 183°C; $[\alpha]_D^{23}$ (c = 4, $CHCl_3$) 132.6.

^{1}H NMR (250 MHz, $CDCl_3$): δ: 1.10 (t, 3H, J = 6.4 Hz, ^{iso}But), 1.15 (t, 3H, J = 6.4 Hz, ^{iso}But), 2.0 (s, 3H, Me), 2.1 (s, 3H, Me), 2.3 (s, 3H, Me), 2.6 (qn/spt, 1H, J = 7.0 Hz, COC*H*Me_2), 3.15 (qn/spt, 1H, J = 6.4 Hz, COC*H*Me_2), 3.35 (dd, 1H, J = 10.1 & 14.6 Hz, H-6), 3.75 (m, 1H, H-5), 4.01 (d(d), 1H, J = 14.6 Hz, H-6′), 4.85 (m, 2H, J = 4.0 & 10.1 Hz, H-1 and H-4), 5.0 (dd, 1H, J = 4.0 & 10.1 Hz, H-2), 5.5 (t, 1H, J = 10.1 Hz, H-3).

^{13}C NMR (100 MHz, $CDCl_3$): δ: 18.3, 18.7, 18.8, 19.6, 20.5, 20.6, 25.6, 33.9, 34.0, 45.1, 69.2, 69.3, 69.6, 70.1, 89.8, 169.4, 170.1, 173.7, 176.3, 181.8.

IR (KBr, cm^{-1}): *v*: 2977, 2934, 2876, 1749, 1712, 1472, 1434, 1371, 1318, 1234, 1151, 1070, 1021, 983, 805, 477.

MS (FAB): 168.0 (100%), 428.1 (85%), 358.0 (68%), 238.0 (31%), 873.2 (12%, M + H), 895.2 (8%, M + Na).

MS (ES^+, Acc. Mass, M + H) Calcd for $C_{40}H_{60}N_2O_{19}$: 873.3869. Obtained: 873.3878.

2,2′,3,3′-Tetra-O-acetyl-6,6′-bis-(N-acetyl,N-cyclohexanecarbonylamino)-4,4′-di-O-cyclohexanecarbonoyl-6,6′dideoxy-α,α-trehalose, ***5***

To a solution of **1** (5.0 g, 8.5 mmol) in pyridine (40 mL) that had been left standing for 1 day was added excess cyclohexanecarbonyl chloride (10 mL, 68 mmol) and DMAP (5 mg). The reaction mixture was left for 4 h with stirring and poured into water (200 mL), and the product extracted into CH_2Cl_2 twice (100 mL). The CH_2Cl_2 solution was dried and concentrated and the residue crystallized from methanol; yield 1.85 g; m.p. 124–125°C; $[\alpha]_D^{23}$ (c = 1, $CHCl_3$) 148.1.

^{1}H NMR (400 MHz, $CDCl_3$): δ: 1.3 (m, 12H, C_6H_{11}), 1.7 (m, 12H, C_6H_{11}), 1.95 (s, 3H, Me), 2.10 (s, 3H, Me), 2.30 (s, 3H, Me), 3.29 (dd, 1H, J = 9.9 & 14.7 Hz, H-6), 3.70 (ddd, 1H, J = 2.1, 9.9 & 10.3 Hz, H-5), 3.99 (dd, H1, J = 2.1 & 14.7 Hz, H-6′), 4.78 (d, 1H, J = 3.8 Hz, H-1), 4.85 (t, 1H, J = 10.3 Hz, H-4), 5.00 (dd, 1H, J = 3.8 & 10.3 Hz, H-2), 5.40 (t, 1H, J = 10.3 Hz, H-3).

^{13}C NMR (63 MHz, $CDCl_3$): δ:20.7, 25.2, 25.3, 25.5, 25.6, 25.8, 26.0, 28.2, 28.7, 28.9, 30.0, 31.0, 43.0, 43.6, 45.1, 69.1, 69.3, 69.7, 69.9, 90.0, 169.5, 170.2, 173.7, 175.4, 180.8.

IR (KBr, cm^{-1}): v: 29.36, 28.58, 1759, 1715, 1694, 1452, 1165, 1140, 1074, 1024, 983.

MS (FAB): 398.1 (100%), 168.0 (96%), 111.0 (84%), 508.2 (37%), 1033.5 (7%, M + H), 1055.4 (6%, M + Na).

MS (ES^+, Acc. Mass, M + H) Calcd for $C_{52}H_{76}N_2O_{19}$: 1033.5121. Found: 1033.5116.

6,6′-Bis-(N-acetylamino)-6,6′-dideoxy-α,α-trehalose, ***6***

To a solution of **1** (2.0 g, 3.4 mmol) in methanol was added sodium methoxide (18.5 mg, 3.4 mmol) and left stirring for 4 h. TLC using ethyl acetate:methanol (2:1) as eluent showed complete conversion to a baseline product. The solution was neutralized by stirring with DOWEX 50W X-8 resin and filtered and the resin washed with methanol. The filtrates and washings were combined, the solvent was removed at reduced pressure, and the residue was recrystallized from acetone/ethanol and obtained as an acetone solvate. After filtration, the crystals desolvated on standing to give a liquid, which was solidified to a white solid by rotary evaporation; yield of title product was 0.95 g (66.4%); m.p. 132–134°C; $[\alpha]_D^{23}$ (c = 1, CH_3OH) 67.05.

^{1}H NMR (250 MHz, $CDCl_3$): δ: 1.83 (s, 3H, Me), 2.96 (t, 1H, J = 9.6 Hz, H-4), 3.24 (m, 3H, H-2,6,6′), 3.52 (t, 1H, J = 9.6 Hz, H-3), 3.69 (ddd, 1H, J = 3.1, 5.5, & 9.6 Hz, H-5), 4.86 (d, 1H, J = 3.8 Hz, H-1), 7.73 (brs, 1H, N*H*$COCH_3$).

^{13}C NMR (63 MHz, $CDCl_3$): δ: 22.5, 70.5, 71.3, 71.6, 72.3, 93.8, 170.1.

IR (KBr, cm^{-1}): v: 3582–3183, 2919, 1638, 1561, 1439, 1382, 1150, 1109, 1043, 991.

Anal. Calcd for $C_{16}H_{28}N_2O_{11}$: C, 45.20; H, 6.64; N, 6.58. Found: C, 45.49; H,6.48; N, 6.70.

6,6-Bis-(1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronyl)-2,2′,3,3′,4,4′-hexa-O-benzyl-α,α-trehalose, **7**

To a stirred solution of 2,2′,3,3′,4,4′-hexa-*O*-benzyl-*α,α*-trehalose, **9**[5,8] (6.00 g, 6.8 mmol), 1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronic acid (5.40 g,

14.3 mmol), and *p*-(dimethylamino)pyridine (17.40 mg, 0.14 mmol) in acetonitrile (20 mL) was added a solution of DCC (2.95 g, 14.3 mmol) in acetonitrile (10 mL) drop-wise over 10 min. After 4 h, the solution was filtered and the filtrate reduced. The syrupy residue was added with stirring to hot diethyl ether and filtered, and the white solid, (1,3-dicyclohexylcarbodiimide-1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronic acid adduct) discarded. The filtrate was chromatographed using a Chromatotron, with ethyl acetate as eluent. The third fraction was collected; evaporated to leave a residue, which was redissolved in diethyl ether; and evaporated to give a white foam. The foam was shown to be a mixture of two compounds, which were separated using TLC with ethyl acetate:petrol (1:1). The less mobile compound, the title compound, was collected as a foam; yield 1.6 g (15%); m.p. 53–56°C; $[\alpha]_D^{24}$ (c = 4, $CHCl_3$) 54.4.

^{1}H NMR (400 MHz, $CDCl_3$): *δ*: 1.97 (s, 3H, Me), 1.98 (s, 3H, Me), 2.00 (s, 3H, Me), 2.01 (s, 3H, Me), 3.41 (m, 1H, H-5 [T]), 3.54 (m, 2H, H-6, 6′ [T]), 4.00 (m, 2H, [G]), 4.16 (m, 2H, [T]), 4.47 (d, 1H, J = 10.9, CH_2Ph), 4.66 (m, 2H, CH_2Ph), 4.83 (m, 2H, CH_2Ph), 4.96 (dd, 1H, CH_2Ph), 5.06 (m, 2H, [G, T]), 5.18 (m, 2H, [G, T]), 5.66 (d, 1H, J = 7.5, H-1 [G]), 7.2–7.4 (m, 15H, Ar) (G = glucose; T = trehalose).

^{13}C NMR (100 MHz, $CDCl_3$): *δ*: 21.5, 21.6, 21.6, 21.7, 69.9, 70.0, 71.2, 71.2, 73.1, 74.1, 74.1, 74.2, 74.2, 76.1, 76.6, 82.5, 92.4, 128.4–129.6, 139.0, 139.3, 139.6, 167.1, 167.7, 170.2, 170.2, 171.0.

IR (KBr, cm^{-1}): *ν*: 2929, 1752, 1670, 1511, 1365, 1245, 1216, 1067, 1035.

MS(FAB): 243.1 (100%), 1593.6 (7%, M + Na), 333.2 (5%).

Anal. Calcd for $C_{82}H_{90}O_{31}$: C, 62.65; H, 5.77. Found: C, 62.46; H, 5.93.

6,6′-Bis-(1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronyl)-α,α-trehalose, **8**

A solution of 6,6-*bis*-(1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronyl)-2,2′,3,3′,4,4′-hexa-*O*-benzyl-*α,α*-trehalose, **7** (2.00 g, 1.3 mmol), in ethanol (25 mL) was hydrogenated over palladium on charcoal (1.05 g, 10%). After 3 days, the reaction mixture was filtered and the filtrate was evaporated at reduced pressure to leave a colorless oil, which was crystallized from ethanol/acetone at –22°C. This solid liquified at rt. Recrystallization from ethanol and diethyl ether produced a hydroscopic solid. Yield of the white solid was 1.25 g (95.3%); m.p. 124°C.

The ^{1}H NMR spectrum was not resolved.

^{13}C NMR (100 MHz, DMSO): *δ*: 21.0, 21.1, 21.2, 21.3, 21.4, 62.6, 62.7, 71.3, 71.7, 71.8, 73.1, 73.7, 73.7, 74.5, 94.9, 95.2, 171.2.

MS (ES^+): 709.6 (100%, M-**35** + Na), 399.8 (35%), 1053.4 (30%, M + Na), 365.8 (32%), 650.9 (31%), 881.6 (15%).

Anal. Calcd for $C_{40}H_{54}O_{31}$: C, 46.60; H, 5.28. Found: C, 46.87; H,5.41.

4,6:4′,6′-Di-O-benzylidene-α,α-trehalose

DMF (60 mL) was dried by refluxing with toluene (60 mL) and rotary evaporation at 60°C to remove toluene and any residue water. *p*-Toluenesulfonic acid monohydrate (0.24 g, 1.4 mmol) was dehydrated by rotary evaporation of a suspension in toluene (60 mL). *α,α*-Trehalose dihydrate (10.00 g, 26.5 mmol) was dried by refluxing in ethanol (60 mL) for 1 h and drying overnight at 60°C. Subsequent weight of anhydrous trehalose was 8.97 g (99%). To a suspension of the anhydrous trehalose in dried DMF (30 mL) was added a solution of *p*-toluenesulfonic acid and (dimethoxymethyl)benzene (4 mL, 26.5 mmol) in dry DMF (30 mL). The mixture was heated at 100°C for 10 min and concentrated at 60°C. More (dimethoxymethyl)benzene (4 mL, 26.5 mmol) was added and the heating procedure repeated. A further amount of (dimethoxymethyl)benzene (1 mL, 6.6 mmol) was added, and the mixture was heated on a steam bath for 10 min to give a colorless solution. The solvent was removed at reduced pressure and the residue crystallized on shaking with toluene and water. Yield of title product was 12.36 g (91%); m.p. 140–145°C (DSC); $[\alpha]_D^{25}$ (c = 3, CH_3OH) 165.2; lit.[33] m.p. 197–198°C (from ethanol); lit[33] $[\alpha]_D$ ($CHCl_3$) 93 and (MeOH) 81.3.[34]

^{1}H NMR (400 MHz, $CDCl_3$): *δ*: 3.45 (t, 1H, *J* = 9.Hz, H-3), 3.59 (dd, 1H, *J* = 3.9 & 9.3 Hz, H-2), 3.68 (t, 1H, *J* = 9.9 Hz, H-6), 3.99 (t, 1H, *J* = 9.3 Hz, H-4), 4.07 (dt, 1H, *J* = 4.8 & 9.3 Hz, H-5), 4.18 (dd, 1H, *J* = 4.8 & 9.9 Hz, H-6′), 4.54 (brs, 2H, O*H*), 5.08 (d, 1H, *J* = 3.9 Hz, H-1), 5.23 (s, 1H, C*H*Ar).

^{13}C NMR (100 MHz, $CDCl_3$): *δ*: 63.8 (C-5), 69.6 (C-6), 71.1 (C-4), 73.4 (C-2), 82.6 (C-3), 96.0 (C-1), 102.7 (C*H*Ar), 127.2, 128.7, 128.9, 129.6, 138.8.

MS (ES^+): 541.0 (100%, M + Na), 119.0 (40%), 160.0 (30%), 371.0 (30%), 573.1 (15%, M + Na + MeOH), 557.1 (5%, M + K).

2,2′,3,3′-Tetra-O-acetyl-4,6:4′,6′-di-O-benzylidene-α,α-trehalose

To a solution of 4,6:4′,6′-di-*O*-benzylidene-*α,α*-trehalose (66.0 g, 12.7 mmol) in pyridine (100 mL) and acetonitrile (100 mL) was added acetic anhydride (80 mL, 0.78 mmol). After stirring for 18 h, the solvent was removed under reduced pressure. The oily residue was precipitated on addition to water with vigorous stirring. The precipitate was filtered and dried; yield of title compound 63.7 g (73%); m.p. 267–268°C (DSC), lit m.p. 246–247°C (from CH_2Cl_2/diethyl ether)[35]; $[\alpha]_D^{24}$ (c = 4, CH_3OH) 188.5, lit $[\alpha]_D$ ($CHCl_3$) value 124.[35]

^{1}H NMR (250 MHz, $CDCl_3$): δ: 2.06 (s, 3H, Me), 2.12 (s, 3H, Me), 3.67 (t, 1H, $J = 9.8$ Hz, H-4), 3.73 (dd, 1H, $J = 9.8$ & 10.4 Hz, H-6), 3.98 (ddd, 1H, $J =$ 4.9, 9.8, & 9.8 Hz, H-5), 4.15 (dd, 1H, $J = 4.9$ & 10.4 Hz, H-6′), 4.99 (dd, 1H, $J = 4.0$ & 9.8 Hz, H-2), 5.33 (d, 1H, $J = 4.0$ Hz, H-1), 5.48 (s, 1H, C*H*Ar), 5.61 (t, 1H, $J = 9.8$ Hz, H-3), 7.33 (m, 3H, Ar), 7.41 (m, 2H, Ar).

^{13}C NMR (63 MHz, $CDCl_3$): δ: 20.7, 20.9, 63.1, 68.6, 69.0, 70.9, 79.0, 93.1, 101.8, 126.2, 128.3, 128.4, 129.2, 136.7, 169.7, 170.4.

IR (KBr, cm^{-1}): ν: 2976, 2939, 2866, 1753, 1374, 1233, 1137, 1097, 1062, 1002, 982.

MS (ES^+): 709.1 (100%, M + Na), 725.1 (12%, M + K).

2,2′,3,3′-Tetra-O-acetyl-α,α-trehalose, ***13***

2,2′,3,3′-Tetra-*O*-acetyl-4,6:4′,6′-di-*O*-benzylidene-*α,α*-trehalose (30.0 g, 43.7 mmol) was dissolved in acetic acid (540 mL) and heated to 95°C, and water (360 mL) was slowly added to the stirred solution. After 20 min at 95°C, the solvent was removed under reduced pressure. Toluene (200 mL) was added to the residue, the solvent was removed under reduced pressure, and toluene (200 mL) again was added, followed by diethyl ether. The mixture was rotary evaporated. The procedure was repeated until the residue became a foam. Crystallization from ethyl acetate yielded 17.05 g (78%) of the title product; m.p. 180–183°C (DSC); $[\alpha]_D^{23}$ (c = 3, CH_3OH) 303.7.

^{1}H NMR (400 MHz, $CDCl_3$): δ: 2.03 (s, 3H, Me), 2.04 (s, 3H, Me), 3.55 (t, 1H, $J = 9.6$ Hz, H-4), 3.66 (dd, 1H, $J = 5.1$ & 12.0 Hz, H-6), 3.73 (dd, 1H, $J = 2.4$ & 12.0 Hz, H-6′), 3.85 (ddd, 1H, $J = 2.4$, 5.1, & 9.6 Hz, H-5), 4.84 (dd, 1H, $J = 3.8$ & 10.3 Hz, H-2), 5.26 (d, 1H, $J = 3.8$ Hz, H-1), 5.36 (dd, 1H, $J = 9.6$ & 10.3 Hz, H-3).

^{13}C NMR (63 MHz, $CDCl_3$): δ: 20.9, 21.0, 62.2, 69.8, 72.1, 74.1, 74.4, 94.3, 171.9, 172.3.

IR (KBr, cm^{-1}): ν: 3564–3341, 2937, 1746, 1438, 1371, 1249, 1133, 1051, 999, 942.

MS (ES^+): 533.0 (100%, M + Na), 491.0 (10%, M-Ac + Na), 549.0 (4%, M + K).

Anal. Calcd for $C_{20}H_{30}O_{15}$: C, 46.87; H, 5.90. Found: C, 46.59; H, 6.04.

2,2′,3,3′-Tetra-O-acetyl-6,6′-bis-(1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronyl)-α,α-trehalose, 10, and 2,2′,3,3′-tetra-O-acetyl-6-(1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronyl)-α,α-trehalose, ***14***

A solution of 1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranuronic acid (3.28 g, 8.6 mmol) in CH_2Cl_2 (20 mL), was dried over anhydrous calcium chloride. After

removal of the drying agent, toluene (50 mL) was added and the solvent was removed at reduced pressure to leave a dried sample of solid 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronic acid. To a solution of this solid with 2,2′,3,3′-tetra-*O*-acetyl-α,α-trehalose, **13** (2.00 g, 3.9 mmol), and *p*-(dimethylamino)pyridine (82 μg) in acetonitrile (30 mL) was slowly added a solution of DCC (1.78 g, 8.6 mmol) in acetonitrile (10 mL). After stirring overnight, the solid product, the DCC adduct, was collected by filtration. More DCC adduct was obtained from the filtrate on addition of methanol (10 mL). After removal of this by-product, the solvent was removed at reduced pressure to leave a residue, which was crystallized from a mixture of CH_2Cl_2 and diethyl ether; yield 3.10 g, m.p. 129–133°C. TLC with ethyl acetate eluent indicated the presence of two products, which were separated by column chromatography using ethyl acetate as eluent. From 400 mg of the mixed product, pseudo-tetrasaccharide (**10**, 170 mg) and pseudo-trisaccharide (**14**, 90 mg) were obtained in pure states; m.p. of **10** was 219–220°C and **14** was 203–205°C.

MS (ES^+) of the unseparated products: 119.0 (100%), 150.9 (35%), 877.1 (15%, **14** + Na), 1221.2 (7%, **10** + Na).

^{1}H NMR of **10** (400 MHz, $CDCl_3$): δ: 1.97 (s, 6H, Me), 1.98 (s, 6H, Me), 2.05 (s, 3H, Me), 2.08 (s, 3H, Me), 3.51 (t, 1H, H-4 [T]), 3.86 (m, 1H, H-5 [T]), 4.22 (m, 2H, H-5 [G], H-6 [T]), 4.39 (dd, 1H, H-6′ [T]), 4.93 (dd, 1H, H-2 [T]), 5.13–5.28 (m, 5H, H-2,3,4 [G], H-3,1 [T]), 5.73 (d, 1H, $J = 7.9$, H-1 [G]).

^{13}C NMR of **10** (100 MHz, $CDCl_3$): δ: 20.4, 20.5, 20.7, 20.9, 61.9, 64.2, 68.7, 69.1, 69.8, 70.1, 70.3, 71.9, 72.8, 72.9, 91.4, 94.4, 166.5, 168.8, 169.2, 169.7, 169.9, 170.0, 172.0.

Acc. Mass (M + NH_4): Calcd for $C_{48}H_{66}NO_{35}$: 1216.3415. Found 1216.3418.

^{1}H NMR of **14** (400 MHz, $CDCl_3$): δ: 1.98 (s, 3H, Me), 1.99 (s, 3H, Me), 2.00 (s, 3H, Me), 2.03 (s, 3H, Me), 2.06 (s, 3H, Me), 2.07 (s, 3H, Me), 2.08 (s, 3H, Me), 2.09 (s, 3H, Me), 3.52 (t, 1H, $J = 9.9$ Hz, H-4 [T_2]), 3.64 (t, 1H, $J = 9.6$ Hz, H-4a [T_1]), 3.69–3.80 (m, 3H, H-5, 6, 6′, [T_1]), 3.92 (m, 1H, H-5, [T_2]), 4.23 (d, 1H, $J = 9.2$ Hz, H-5 [G]), 4.25 (dd, 1H, $J = 5.4$ & 11.0 Hz, H-6 [T_2]), 4.38 (dd, 1H, $J = 2.4$ & 11.0 Hz, H-6′ [T_2]), 4.89 (dd, 2H, $J = 4.0$ & 7.2 Hz, H-2 [T_1orT_2]), 4.90 (dd, 2H, $J = 3.6$ & 7.2 Hz, H-2 [T_1orT_2]), 5.12 (t, 1H, $J = 7.8$ Hz, H-2 [G]), 5.18 (d, 1H, $J = 4.0$ Hz, H-1 [T_1orT_2]), 5.19 (d, 1H, $J = 4.0$ Hz, H-1 [T_1orT_2]), 5.22–5.30 (m, 4H, H-3,4 [G], H-3 [T_1&T_2]), 5.74 (d, 1H, $J =$ 7.8 Hz, H-1 [G]).

^{13}C NMR of **14** (100 MHz, $CDCl_3$): δ: 20.4, 20.5, 20.6, 20.7, 20.9, 62.0 (C-6 [T_1]), 64.2 (C-6 [T_2]), 68.8, 69.9, 70.1, 70.3, 71.9, 72.5, 72.8, 73.4, 91.5 (C-1 [G]), 92.4 (C-1 [T]), 94.4 (C-1 [T]), 166.5, 168.8, 169.2, 169.7, 169.9, 170.2, 172.0, 172.1.

MS (ES^+) of **14**: 876.5 (100%, M + Na), 871.5 (76%, M + NH_3), 794.5 (52%), 674.5 (38%), 428.5 (24%).

Anal. Calcd for $C_{34}H_{46}O_{25}$: C, 47.78; H, 5.43. Found: C, 48.05; H, 5.64.

2,2′,3,3′-Tetra-O-acetyl-6,6′-bis-(1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronyl)-4,4′-di-O-isobutyryl-α,α-trehalose, 11, and 2,2′,3,3′-tetra-O-acetyl-6-(1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronyl)-4,4′,6′-tri-O-isobutyryl-α,α-trehalose, ***15***

To a solution of the unseparated products of the above reaction, compounds **10** and **14** (1.00 g) in pyridine (4 mL) and acetonitrile (4 mL), was added isobutoyl chloride (2.5 mL, large excess). After stirring for 18 h the solvent was removed under reduced pressure to leave an oil, which was poured into water (30 mL), extracted into CH_2Cl_2 (10 mL), dried, filtered over activated charcoal, and crystallized from $CH_2Cl_2$and petroleum ether (40–60°C). Yield of the combined products was 0.23 g (20.6%), m.p. 109–113°C. Compounds **11** and **15** (55 mg) were separated using column chromatography, using ethyl acetate and petrol ether 40–60°C (2:1) as the eluent. The lower running spot was dissolved in ethyl acetate and crystallized on addition of diethyl ether; yield of compound **11** (24 mg); m.p. (ethyl acetate/diethyl ether) 93–94°C.

^{1}H NMR of **11** (400 MHz, $CDCl_3$): δ: 1.07 (d, 3H, $J = 6.8$ Hz, $O_2CCH(CH_3)_2$), 1.09 (d, 3H, $J = 2.7$ Hz, $O_2CCH(CH_3)_2$), 2.48 (m, 1H, $J = 2.7$ & 6.8Hz, $O_2CCH(CH_3)_2$), 3.93 (m, 2H, H-5, H-6 [T]), 4.11 (d(d), 1H, $J = 10.3$ Hz, H-6′ [T]), 4.22 (d, 1H, $J = 9.2$ Hz, H-5 [G]), 4.88 (t, 1H, $J = 9.9$Hz, H-4 [T]), 4.97 (dd, 1H, $J = 4.1$ & 9.9 Hz, H-2 [T]), 5.15 (t, 1H, $J = 8.0$ Hz, H-2 [G]), 5.20–5.27 (m, 3H, H-3, H-4 [G], H-1 [T]), 5.47 (t, 1H, $J = 9.9$ Hz, H-3 [T]), 5.74 (d, 1H, $J = 8.0$ Hz, H-1 [G]).

^{13}C NMR of **11** (100 MHz, $CDCl_3$): δ: 18.6, 18.7, 20.4, 20.4, 20.5, 20.5, 20.6, 20.7, 33.8, 63.6 (C-6 [T]), 68.1 (C-4/5 [T]), 68.2 (C-4/5 [T]), 68.6 (C-3/4 [G]), 69.5 (C-3 [T]), 69.9 (C-2 [T]), 70.1 (C-2 [G]), 71.9 (C-3/4 [G]), 72.7 (C-5 [G]), 90.7 (C-1 [T]), 91.5 (C-1 [G]), 166.1 (C-6 [G]), 168.8, 169.1, 169.4, 169.6, 169.9, 169.9, 175.6.

MS (ES^+) of **14**: 119.0 (100%), 151.1 (45%), 1087.3 (40%), 1361.2 (20%, M + Na).

Acc. Mass (M + NH_4) of **14**: Calcd 1356.4253. Found 1356.4284

^{1}H NMR of **15** (400 MHz, $CDCl_3$): δ: 1.07–1.12 (m, 18H, $CH(CH_3)_2$), 1.97 (s, 9H, 3 × Me), 1.98 (s, 3H, Me), 2.00 (s, 3H, Me), 2.03 (s, 3H, Me), 2.04 (s, 3H, Me), 2.07 (s, 3H, Me), 2.46–2.54 (m, 3H, $CH(CH_3)_2$), 3.93–4.02 (m, 4H), 4.11 (m, 2H), 4.24 (d, 1H, $J = 9.6$ Hz, H-5 [G]), 4.91 (t, 1H, $J = 9.6$ Hz), 4.95–5.04 (m,

3H), 5.17 (dd, $J = 7.9$ & 8.9 Hz), 5.20–5.29 (m, 4H), 5.49 (t, 2H, $J = 9.2$ Hz, H-3 [T & T′]), 6.76 (d, 1H, $J = 7.9$ Hz, H-1 [G]).

^{13}C NMR of **15** (100 MHz, $CDCl_3$): δ: 18.7, 18.7, 18.8, 18.9, 20.5, 20.6, 20.6, 20.7, 29.7, 33.7, 33.8, 61.5, 63.6, 68.1, 68.2, 68.4, 68.6, 69.7, 69.8, 69.9, 70.2, 72.0, 72.7, 91.5, 166.1, 168.8, 169.2, 169.5, 169.6, 169.8, 169.9, 169.9, 175.5, 175.6, 176.6.

Anal. Calcd for $C_{46}H_{62}O_{28}$: C, 51.97; H, 5.88. Found: C, 52.13; H, 5.93.

2,2′,3,3′-Tetra-O-acetyl-6,6′-bis-(1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronyl)-4,4′-di-O-cyclohexanecarbonyl-α,α-trehalose, 12, and 2,2′,3,3′-tetra-O-acetyl-6-(1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronyl)-4,4′,6′-tri-O-cyclohexanecarbonyl-α,α-trehalose, ***16***

To a solution of the unseparated compounds **10** and **14** (1.00 g) in pyridine (4 mL) and acetonitrile (5 mL) was added cyclohexanecarbonyl chloride (2 mL, large excess). After stirring for 18 h, the solvent was removed under reduced pressure to leave an oil, which was poured into water (20 mL), extracted into CH_2Cl_2 (10 mL), dried, and filtered over activated charcoal. The solvent was removed under reduced pressure and the residue was crystallized from diethyl ether and petroleum ether (40–60°C). Yield of combined title products 0.73 g (61.7%). TLC with ethyl acetate and petroleum ether (40–60°C) eluent provided good separation of the products. Column chromatography, using the same eluent system, of the white solid (400 mg) yielded the pseudotrisaccharide (**16**, 47.3 mg) as the higher running TLC spot and the pseudotetrasaccharide (**12**, 181.7 mg) as the lower running TLC spot.

^{1}H NMR of **12** (400 MHz, $CDCl_3$): δ: 1.16–1.34 (m, 5H, $C_6H_{11}CO_2$), 1.59–1.80 (m, 6H, $C_6H_{11}CO_2$), 1.96 (s, 3H, Me), 1.97 (s, 3H, Me), 1.98 (s, 3H, Me), 2.00 (s, 3H, Me), 2.03 (s, 3H, Me), 2.07 (s, 3H, Me), 3.94 (m, 2H, H-5, H-6 [T]), 4.12 (d(d), 1H, $J = 10.6$ Hz, H-6′ [T]), 4.24 (d, 1H, $J = 9.2$ Hz, H-5 [G]), 4.90 (t, 1H, $J = 9.6$ Hz, H-4 [T]), 4.98 (dd, 1H, $J = 4.1$ Hz, H-2 [T]), 5.17 (t, 1H, $J = 8.2$ Hz, H-2 [G]), 5.24 (m, 3H, H-3, H-4 [G], H-1 [T]), 5.48 (t, 1H, $J = 9.9$ Hz, H-3 [T]), 5.75 (d, 1H, $J = 7.9$ Hz, H-1 [G]).

^{13}C NMR of **12** (63 MHz, $CDCl_3$): δ: 20.5, 20.6, 20.7, 20.8, 25.3, 25,5, 28.7, 42.9, 63.6 (C-6 [T]), 68.1 (C-4 [T]), 68.2 (C-5 [T]), 68.7 (C-3/4 [G]), 69.5 (C-3 [T]), 70.0 (C-2 [T]), 70.1 (C-2 [G]), 72.0 (C-3/4 [G]), 72.7 (C-5 [G]), 91.0 (C-1 [T]), 91.5 (C-1 [G]), 166.1 (C-6 [G]), 168.9, 169.2, 169.5, 169.7, 169.9, 170.0, 174.6.

MS (ES^+) of **12**: 293.0 (100%), 261.0 (75%), 499.1 (35%), 539.2 (22%), 1441.3 (15%, M + Na).

Acc. Mass (M + NH_4) of **12**: Calcd for $C_{62}H_{82}O_{37}$: 1436.4879. Found 1436.4885.

^{1}H NMR of **16** (400 MHz, $CDCl_3$): δ: 1.16–1.40 (m, 15H), 1.52 (s, 3H), 1.60–1.81 (m, 15H), 1.97 (s, 3H, Me), 1.97 (s, 6H, 2 × Me), 1.99 (s, 3H, Me), 2.00 (s, 3H, Me), 2.04 (s, 3H, 2 × Me), 2.08 (s, 3H, Me), 3.91–4.01 (m, 4H, H-5, 6 [T & T′]), 4.12 (m, 2H, H-6′ [T & T′]), 4.25 (d, 1H, J = 9.2 Hz, H-5 [G]), 4.92 (t, 1H, J = 9.6, H-4 [T or T′]), 4.97–5.06 (m, 3H, H-2 [T&T′], H-4 [T or T′]), 5.18 (t, 1H, J = 7.8 Hz, H-2 [G]), 5.16–5.30 (m, 4H, H-3, 4 [G], H-1 [T & T′]), 5.45–5.50 (t, 2H, J = 9.3 Hz, H-3 [T & T′]), 5.77 (d, 1H, J = 7.5 Hz, H-1 [G]).

^{13}C NMR of **16** (63 MHz, $CDCl_3$): δ: 20.5, 20.6, 20.7, 20.8, 25.3, 25.4, 25.4, 25.5, 25.7, 28.8, 29.0, 42.9, 61.3, 63.6, 67.8, 68.1, 68.1, 68.4, 68.7, 69.7, 69.8, 70.0, 70.2, 72.1, 72.7, 91.5, 91.6, 91.8, 166.1, 168.9, 169.2, 169.5, 169.6, 169.7, 169.8, 169.9, 170.0, 174.5, 174.6, 175.6.

MS (ES^+) of **16**: 1124.7 (100%), 1201.7 (35%), 1206.6 (25%, M + Na).

Anal. Calcd for $C_{55}H_{74}O_{28}$: C, 55.83; H, 6.30. Found: C, 55.69; H, 6.43.

6,6′-Di-O-trityl-α,α-trehalose

6,6′-Di-*O*-trityl-*α,α*-trehalose was obtained using a modification of the method reported by Brederick.[36] Trityl chloride (17.0 g, 61 mmol) was slowly added to a solution of anhydrous trehalose (10.5 g, 31 mmol) in pyridine (40 mL). The reaction mixture was stirred at 40°C for 36 h and evaporated under reduced pressure to 2 mL, and the residue poured into cold water with stirring. The pale yellow precipitate was washed with water and dried at 70°C. Hot methanol was added to the solid and stirred for 5 min to form a slurry, which was filtered and the solid dried at 70°C for 12 h. Yield of product was 19.9 g (83%); m.p. 191–193°C: lit.[26] value 278–281°C; $[\alpha]_D^{24}$ (c = 2, DMSO) +100.1: lit.[26] value $[\alpha]_D^{19}$ (pyridine) +62.8.

^{1}H NMR (250 MHz, DMSO-d_6): δ: 3.04 (m, 1H, H-6), 3.20 (m, 2H, H-4, 6′), 3.40 (dd, 1H, J = 3.1, 9.3, H-2), 6.63 (t, 1H, J = 9.3, H-3), 4.05 (m, 1H, H-5), 5.15 (d, 1H, J = 3.1, H-1), 7.23–7.35 (m, 9H, *m, p*−Ar), 7.45 (d, 6H, J = 7.3, *o*−Ar).

^{13}C NMR (63 MHz, DMSO-d_6): δ: 63.5, 70.6, 71.1, 71.8, 73.5, 85.5, 126.8, 126.9, 127.0, 127.7, 127.9, 128.3, 128.4, 128.5, 128.6, 144.1.

2,2′,3,3′,4,4′-Hexa-O-acetyl-6,6′-di-O-trityl-α,α-trehalose

Acetic anhydride (20 mL, 196 mmol) was slowly added to a solution of 6,6′-di-*O*-trityl-*α,α*-trehalose (19.86 g, 24 mmol) in pyridine (60 mL) to maintain the temperature below 30°C. The mixture was stirred for 4 h and evaporated and the residue poured into ice water with vigorous stirring. The pale brown precipitate was washed with water (150 mL) and stirred with methanol for 10 min at 60°C. The solid obtained on cooling was collected and dried overnight

at 60°C; yield 22.11 g (86%); m.p. 248–250°C: lit[36] value 245–250°C; $[\alpha]_D^{23}$ (c = 6, $CHCl_3$) 251.5; lit.[36] value [α] ($CHCl_3$)+114.7.

^{1}H NMR (250 MHz, $CDCl_3$): δ: 1.74 (s, 6H), 1.89 (s, 6H), 1.99 (s, 6H), 3.10 (m, 2H, H-6,6′), 4.12 (m, 1H, H-5), 5.14 (t, 1H, J = 9.8 Hz, H-4), 5.19 (dd, 2H, J = 3.7 Hz, H-2), 5.45 (t, 1H, J = 9.8 Hz, H-3), 5.46 (d, 1H, J = 3.7 Hz, H-1), 7.17–7.42 (m, 15H, 3 × Ph).

^{13}C NMR (63 MHz, $CDCl_3$): δ: 20.5, 20.6, 20.8, 61.8, 68.9, 69.7, 69.8, 70.6, 86.6, 92.8, 127.1, 127.3, 127.9, 128.6, 143.4, 169.2, 169.7, 170.2.

2,2′,3,3′,4,4′-Hexa-O-acetyl-α,α-trehalose, ***18***

To a solution of 2,2′,3,3′,4,4′-hexa-*O*-acetyl-6,6′-di-*O*-trityl-α,α-trehalose (45 g, 42 mmol) in dichloromethane (200 mL) was added freshly ground ferric chloride hexahydrate (30 g, 111 mmol) with stirring. After 1 h, water (200 mL) was added, the mixture was stirred for 20 min, and the organic layer collected. This was washed with water (2 × 150 mL), dried over anhydrous magnesium sulphate, and evaporated. Fractional recrystallization of the residue initially from methanol, to remove the trityl coproduct, and finally from methyl *t*-butyl ether at 0°C, gave the title product, 12 g (49%); m.p. 102°C: lit.[36] value 93–96°C; $[\alpha]_D^{24}$ (c = 4, $CHCl_3$) 253; lit[36] value $[\alpha]_D^{19}$ ($CHCl_3$)+158.8.

^{1}H NMR (250 MHz, $CDCl_3$): δ: 2.03 (s, 3H, Me), 2.07 (s, 3H, Me), 2.08 (s, 3H, Me), 3.57 (m, 2H, H-6,6′), 3.94 (ddd, 1H, J = 3.1, 4.3, & 10.1 Hz, H-5), 4.97 (dd, 1H, J = 3.97 & 10.1 Hz, H-2), 5.00 (dd, 1H, J = 9.5 Hz, 10.1, H-4), 5.30 (d, 1H, J = 3.97 Hz, H-1), 5.52 (dd, 1H, J = 9.5 & 10.1 Hz, H-3).

^{13}C NMR (63 MHz, $CDCl_3$): δ: 20.7, 61.0, 60.8, 69.8, 70.2, 70.6, 93.2, 170.0, 170.2, 170.3.

IR (KBr): *v*: 3498, 2951, 1757, 1431, 1371, 1227, 1130, 1043, 984, 953.

MS (ES^+): 617.1 (100%, M + Na), 131.2 (20%), 428.3 (15%), 1211.2 (8%, 2M + Na).

Anal. Calcd for $C_{24}H_{34}O_{17}$: C, 48.48; H, 5.76. Found: C, 48.65; H, 5.90.

2,2′,3,3′,4,4′-Hexa-O-acetyl-6,6′-di-O-succinyl-α,α-trehalose, ***19***

A solution of 2,2′,3,3′,4,4′-hexa-*O*-acetyl-α,α-trehalose, **18** (2.50 g, 4.2 mmol), and succinic anhydride (3.25 g, 32.5 mmol) in pyridine (20 ml) was stirred for 24 h, poured into water (100 mL), and acidified with concentrated hydrochloric acid (10 mL). The aqueous solution was extracted with dichloromethane (2 × 50 ml). The combined organic solutions were dried, the solvent was removed under reduced pressure, and the residue recrystallized from diethyl ether, yield 1.67 g (50%); m.p. 142–145°C (DSC); $[\alpha]_D^{24}$ (c = 2.5, $CHCl_3$) 131.3.

^{1}H NMR (250 MHz, $CDCl_3$): δ: 2.00 (s, 3H, Me), 2.02 (s, 3H, Me), 2.10 (s, 3H, Me), 2.35–2.85 (m, 4H, $O_2CCH_2CH_2CO_2$), 3.74 (dd, 1H, $J = 1.8$ & 11.9 Hz, H-6′), 4.04 (ddd, 1H, $J = 1.8$, 8.9, & 9.7 Hz, H-5), 4.68 (dd, 1H, $J = 8.9$ & 11.9 Hz, H-6), 4.92 (t, 1H, $J = 9.7$ Hz, H-4) 5.01 (dd, 1H, $J = 3.4$ & 9.7 Hz, H-2), 5.27 (d, 1H, $J = 3.4$ Hz, H=1), 5.48 (t, 1H, $J = 9.7$ Hz, H-3).

^{13}C NMR (63 MHz, $CDCl_3$): δ: 15.3, 20.6, 20.7, 28.4, 29.1, 43.5, 61.6, 65.9, 68.0, 69.7, 69.8, 90.9, 169.7, 170.0, 172.1, 179.4.

IR (KBr): ν: 3612-3347, 2964, 1761, 1740, 1718, 1436, 1371, 1245, 1168, 1042, 984, 956.

MS (ES^+): 817.1 (100%, M + Na), 833.1 (10%, M + K).

Anal. Calcd for $C_{32}H_{42}O_{23}$: C, 48.31; H, 5.33. Found: C, 48.51; H, 5.42.

2,2′,3,3′,4,4′-Hexa-O-acetyl-6,6′-bis-(1,2,3,4-tetra-O-acetyl-6-O-succinyl-β-D-glucopyranuronyl)-α,α-trehalose, ***17***

2,2′,3,3′,4,4′-Hexa-*O*-acetyl-6,6′-di-*O*-succinyl-*α,α*-trehalose, **19** (1.00 g, 1.3 mmol), 1,2,3,4-tetra-*O*-acetyl-*β*-D-glucopyranose (0.99 g, 2.7 mmol), and *p*-(dimethylamino)pyridine (4 mg, 0.03 mmol) were dissolved in acetonitrile (10 mL). To this mixture was slowly added a solution of DCC (0.55 g, 2.6 mmol) in acetonitrile (10 mL) with stirring. After 18 h the solution was filtered to remove the 1,3-dicyclohexylcarbodiimide adduct and the filtrate reduced. An impure sample of the title compound, contaminated with one other compound, was obtained on crystallization of the residue from aqueous methanol. Further purification was achieved by column chromatography using ethyl acetate:petrol ether (40–60°C) (2:1) as eluent. The title compound was the more mobile of the two components in the mixture; yield 200 mg (11%); m.p. 113–115°C.

^{1}H NMR (250 MHz, $CDCl_3$): δ: 1.99 (s, 3H, Me), 2.02 (s, 9H, 3 × Me), 2.03 (s, 3H, Me), 2.08 (s, 3H, Me), 2.11 (s, 3H, Me), 2.65 (br.s, 4H, $O_2CCH_2CH_2CO_2$), 3.85 (m, 1H, H-5 [G]), 4.03 (d, 1h, $J = 12.8$Hz, H-6′ [T]), 4.17 (m, 1H, H-5 [T]), 4.17 (d(d), 1H, H-6′ [G]), 4.24 (m, 2H, H-6 [G], H-6 [T]), 5.03 (m, 2H, H-2, 4 [T]), 5.12 (t, 2H, $J = 8.2$ Hz, H-2, 4 [G]), 5.22 (t, 1H, $J = 9.2$ Hz, H-3 [G]), 5.29 (d, 1H, $J = 3.7$ Hz, H-1 [T]), 5.48 (dd, 1H, $J = 8.24$ & 10.1 Hz, H-3 [T]), 5.70 (d, 1H, $J = 8.2$ Hz, H-1 [G]).

^{13}C NMR (63 MHz, $CDCl_3$): δ: 20.4, 20.6, 20.8, 28.6, 28.7, 61.8 (C-6, [T]+[G]), 67.8 (C-4, [G]), 68.2 (C-5, [T]), 68.6 (C-4, [T]), 69.8 (C-2, [T]), 70.0 (C-3, [T]), 70.3 (C-2, [G]), 72.7 (C-5, [G]), 72.8 (C-3, [G]), 91.7 (C-1, [G]), 92.1 (C-1, [T]), 169.0, 169.2, 169.4, 169.6, 169.7, 170.0, 170.1, 171.8, 171.9.

MS (FAB): 269.1 (100%), 229.1 (52%), 209.0 (46%), 557.1 (34%), 335.1 (30%), 719.1 (25%), 431.1 (20%), 845.1 (16%), 1477.4 (15%, M + Na).

Anal. Calcd for $C_{60}H_{70}O_{41}$: C, 49.55; H, 4.85. Found: C, 49.74; H, 4.77.

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