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Note

New access to *C*-disaccharide analogs of α,α-trehalose using an aqueous hetero Diels–Alder reaction

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Abstract

C-disaccharide analogs of trehalose were prepared using an aqueous Diels–Alder reaction as a key step. The resulting major stereoisomer was shown by NMR spectroscopy analysis to have the correct (α, α') stereochemistry of trehalose. © 1997 Elsevier Science Ltd.

Keywords: Trehalose; Trehalase inhibitors; Aqueous hetero Diels–Alder reactions; *C*-Glycoside

There is currently a great deal of interest in the synthesis of trehalose analogs as trehalase inhibitors [1] or biological probes of the enzyme mechanism [2]. Trehalase is the enzyme involved in the catabolism into glucose of the non-reducing α,α-trehalose disaccharide, a key storage carbohydrate in certain insects, fungi, and bacteria, and therefore, inhibitors of its action should have interesting properties as, for instance, insecticides or antibiotics.

In the early seventies, it was demonstrated from results of this laboratory [3] that carbohydrate-derived buta-1,3-dienyl ethers are good precursors for the preparation of oligosaccharides. Later, buta-1,3-dienyl glycosides of unprotected sugars were used to study aqueous Diels–Alder reactions [4]. More recently, we have shown that despite the very low concentration in water of the non-hydrated carbonyl group, the aqueous hetero Diels–Alder reaction is even possible using directly the very cheap, commercially available, aqueous solution of glyoxylic acid as dienophile to give, in good yields, valuable compounds such as substituted dihydropyrans and α-hydroxy-γ-lactones. In particular, this methodology allowed us to prepare diverse ulosonic acids such as KDO or sialic acid analogs [5]. We now report the extension of this work to obtain *C*-disaccharide analogs of trehalose, the key step of our synthesis being the aqueous hetero Diels–Alder reaction between the sodium salt of glyoxylic acid and the water-soluble diene derivative **3**.

Horton and Miyake [6] reported that the reaction of α-D-glucopyranose peracetate with (*E*)-penta-2,4-dienyltrimethylsilane afforded, under Lewis acid catalysis, an inseparable 4:1 mixture of 5-(2,3,4,6-tetra-*O*-acetyl-α and β-D-glucopyranosyl)-(*E*)-1,3-pentadiene (**2**) in 33% yield. Thus, to obtain diene **2** free from the β anomer, we decided to prepare it by a Wittig reaction from anomerically pure α-aldehyde **1** [7]. Condensation of (2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-ethanal (**1**) with allylidenetriphenylphosphorane afforded in 64% isolated yield a mix-

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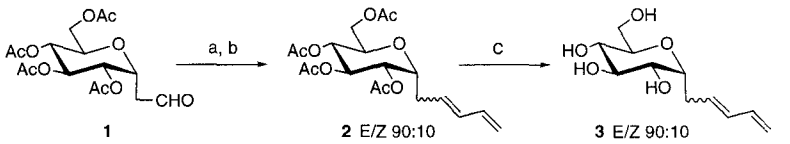
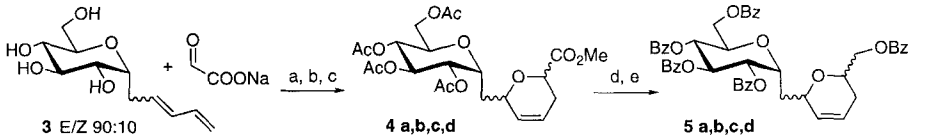
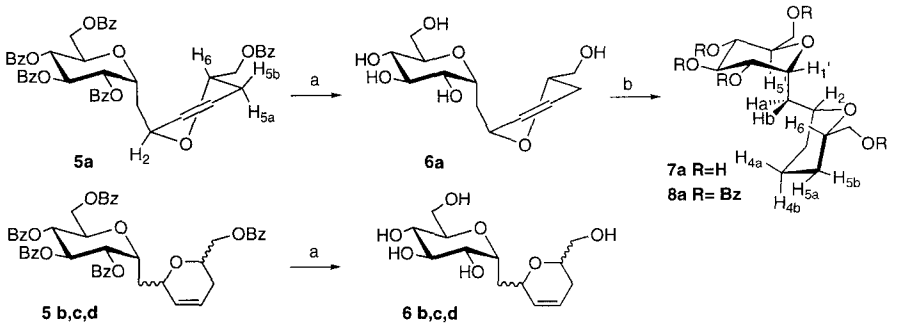


Scheme 1. a, $Ph_3P{=}CH{-}CH{=}CH_2$, THF, −78 °C; b, I_2, $h\nu$, CH_2Cl_2; c, MeOH–Et_3N–H_2O.



Scheme 2. a, H_2O, 140 °C, 48 h; b, MeOH, Dowex-50 (H^+); c, Ac_2O–pyridine; d, $LiBH_4$, *i*PrOH–THF; e, BzCl–pyridine.



Scheme 3. a, NaOMe, MeOH; b, H_2, Pd/C, MeOH.

ture of *E* and *Z* α-diene **2** in a 6:4 ratio. Then, isomerization with a catalytic amount of iodine under light gave a 9:1 *E/Z* mixture in 98% isolated yield. The residual inseparable amount of *Z* isomer was not problematic since this compound did not react in a Diels–Alder reaction and was easily separated after the reaction. Diene **2** was then quantitatively deacetylated under standard conditions (MeOH, Et_3N, H_2O) to obtain the water-soluble α-D-*C*-dienyl glucoside **3** (Scheme 1).

The Diels–Alder reaction of **3** with glyoxylic acid sodium salt (5 equiv) was performed in water at 140 °C in a screw-capped tube. Under these conditions, the cycloaddition was complete within 48 h. We found that, if the cycloadducts were not purified from the excess of glyoxylic acid at this stage, the isolated yields of subsequent reactions were low, due to the formation of very polar compounds, presumably acetals arising from the reaction between the glycosidic moiety of the cycloadducts and glyoxylic acid. So, the mixture of stereoisomers was purified by Dowex-1 (formiate form) chromatography, then esterified by methanol in the presence of Dowex-50 (H^+ form), and finally acetylated. ^{13}C NMR analysis of the mixture showed the presence of four diastereoisomers in the proportions 41:24:21:14. The mixture was then reduced to 6-hydroxymethyl derivatives and benzoylated (Scheme 2). At this stage, the major diastereoisomer **5a** could be easily isolated from the mixture by flash chromatography. The absence of NOE between H-6 and H-2 (pyran numbering [1]) allowed us to attribute a *trans* configuration to **5a** in a half chair conformation [8]. Furthermore, the magnitude of the $J_{5a,6}$ and $J_{5b,6}$ coupling constants indicates a pseudo axial position for H-6, dictating a $^{O}H_5$ conformation for an α-D configuration or a $^{5}H_{O}$ conformation for an α-L configuration. Then, **5a** and the mixture of **5b,c,d** were debenzoylated to afford, respectively, the water-soluble tetrahydropyrans **6a** and **6b,c,d**. Reduction of the double bond of **6a** carried out by hydrogenation over Pd/C in methanol afforded pure **7a** (NMR analysis) fully characterized as its benzoylated derivative **8a** (Scheme 3). The stereo-

[1] Numbering referred to sugar numbering for the glucose moiety and to pyran numbering for the new built pyran moiety.

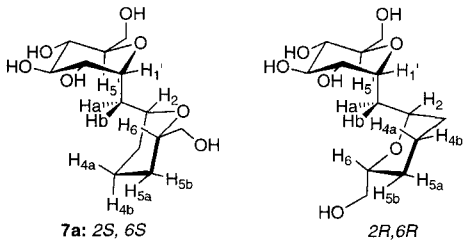


Fig. 1. The two possible diastereoisomers (2*S*,6*S*) and (2*R*,6*R*) for **7**.

chemistry of **7a** was established by NOESY experiments. NOEs between H-2 and H-5′, and H-1′ and H-6 implied a 2*S*,6*S* configuration for the pyran unit (a conformation for the 2*R*,6*R* diastereoisomer satisfying these two strong NOEs should give rise to a strong effect between H-4a and H-1′, see Fig. 1). From these data, and from the ^{1}H interannular methylene bridge pattern, we could conclude that this analog preferentially adopts a relative conformation for the two rings very close to the one observed for α,α-*C*-trehalose [9]. It is noteworthy that the major diastereoisomer arises from an attack onto the *Re* face (related to the prochiral C-4 of the pentadienyl unit), that is the same side as compared to the reaction with the related butadienyl-α-D-glycoside (*O*-glycoside) with various dienophiles [10], indicating probably a similar conformation for the two starting dienes (*C*- or *O*-linked).

The inhibitory activity of **6a**, **6b**,**c**,**d**, and **7a** was assayed on porcine kidney trehalase. The mixture **6b**,**c**,**d** is not an inhibitor, while **6a** and **7a** are very weak inhibitors with IC_{50} values higher than 47 mM, the Km value of this enzyme being 3 mM.

1. Experimental

General methods.—All moisture-sensitive reactions were performed under a nitrogen atmosphere using oven-dried glassware. All solvents were dried over standard drying agents [11] and freshly distilled prior to use. Flash column chromatography [12] was performed on Silica Gel 60A C.C. (6–35 μ). Reactions were monitored by TLC on Silica Gel 60 F_{254} with detection by charring with sulfuric acid. Melting points were determined with a capillary apparatus and are uncorrected. Optical rotations were measured at 20 ± 2 °C. NMR spectra were recorded at room temperature with Bruker AC 200, AC 250, or AM 400 spectrometers. Elemental analyses were performed at the Service Central de Microanalyse du C.N.R.S. (Gif sur Yvette, France).

*5-(2,3,4,6-Tetra-*O*-acetyl-*α*-*D*-glucopyranosyl)-(*E*)- and -(*Z*)-1,3-pentadiene* (**2**).—To a solution of allyltriphenylphosphonium bromide (5.3 g, 13.9 mmol) in THF (70 mL) was added at −78 °C a solution of *n*BuLi (1.6 M, 13.9 mmol) in hexane over a 5 min period. The mixture was allowed to warm to room temperature for 2 h. The allylidenetriphenylphosphorane solution was then transferred dropwise under nitrogen by standard syringe techniques to a stirred, cooled (−78 °C) solution of aldehyde **1** (4.0 g, 10.7 mmol) in THF (100 mL) over a 30 min period. After an additional 2 h, the mixture was allowed to warm to −30 °C in 2 h, then poured into 150 mL of a 5% KH_2PO_4 aq soln and extracted with Et_2O (3 × 150 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. Flash chromatography (5:2 hexane–EtOAc) of the residue gave 2.7 g (64%) of the diene **2** as a 6:4 *E*/*Z* mixture (NMR analysis). Iodine (51 mg, 0.2 mmol) was added to the 6:4 mixture of (*E*)- and (*Z*)-**2** (2.7 g, 6.7 mmol) in CH_2Cl_2 (7 mL). The solution was irradiated with a tungsten lamp (d = 20 cm) at refluxing temperature for 3 h. The mixture was concentrated and purified by flash chromatography (5:2 hexane–EtOAc) to afford 2.6 g (98%) of the diene (*E*)-**2** as a white solid, contaminated by 10% of (*Z*)-**2**. IR (KBr): ν 2990, 1741, 1434, 1371, 1232, 1097, and 1034 cm^{-1}; the NMR parameters for the separated products were extracted from the spectra of the mixture. ^{1}H NMR data of (*E*)-**2** were in agreement with that reported in the literature [6]. (*E*)-**2** (^{13}C, 62.9 MHz, $CDCl_3$): δ 20.7 ($COCH_3$), 29.4 (C-5), 62.2 (C-6′), 68.8 and 70.2 (C-2′, C-3′, C-4′, and C-5′), 71.9 (C-1′), 116.3 (C-1), 128.4, 133.8, and 136.5 (C-2, C-3, and C-4), 169.5, 169.6, 170.1, and 170.6 ($COCH_3$). (*Z*)-**2** (^{13}C, 62.9 MHz, $CDCl_3$), selected data: δ 118.5 (C-1), 125.8, 131.3, and 131.4 (C-2, C-3, and C-4). Anal. Calcd for $C_{19}H_{26}O_9$: C, 57.26; H, 6.58; O, 36.16. Found: C, 57.39; H, 6.41; O, 36.15.

5-(α-D-Glucopyranosyl)-(E)- and -(Z)-1,3-pentadiene (**3**).—A suspension of the peracetylated diene **2** (2.6 g, 6.5 mmol) in 8:1:1 MeOH–H_2O–Et_3N (65 mL) was stirred overnight at room temperature. The resultant clear solution was coevaporated with toluene and water under reduced pressure several times until the odorless solution (free from triethylammonium salts) reached a constant weight. Pure diene **3** was obtained in quantitative yield (1.5 g) as a 9:1 *E*/*Z* mixture. IR (KBr): ν 3396, 2922, 1651, 1450, 1384,

1238, 1096, 1056, and 1008 cm^{-1}; NMR data: (*E*)-**3** (^1H, 250 MHz, CD_3OD): δ 2.23–2.49 (m, 2 H, 2 H-5), 3.10–3.20 (m, H-4′ and CH_3OD), 3.25–3.56 (m, 4 H, H-5′, H-2′, H-3′, and H-6′a), 3.61 (dd, 1 H, $J_{6'a,6'b}$ 11.5, $J_{5',6'b}$ 2.6 Hz, H-6′b), 3.81 (dt, 1 H, $J_{1',2'}$ 5.0, $J_{1',5a} = J_{1',5b} = 10.0$ Hz, H-1′), 4.82 (dd, 1 H, $J_{1a,1b}$ 1.5, $J_{1a,2}$ 10.0 Hz, H-1a), 4.95 (dd, 1 H, $J_{1b,2}$ 16.6 Hz, H-1b), 5.63 (dt, $J_{3,4}$ 15.0, $J_{4,5a} = J_{4,5b} = 7.0$ Hz, H-4), 6.02 (br dd, 1 H, $J_{2,3}$ 10.0 Hz), 6.21 (dt, 1 H, H-2); (^{13}C, 62.9 MHz, CD_3OD): δ 29.2 (C-5), 62.9 (C-6′), 72.1, 72.9, 74.5, and 75.1 (C-2′, C-3′, C-4′, and C-5′), 77.2 (C-1′), 115.4 (C-1), 132.4, 134.1, and 138.5 (C-2, C-3, and C-4). Anal. Calcd for $C_{11}H_{18}O_5$: C, 57.38; H, 7.88; O, 34.74. Found.: C, 57.05; H, 7.71; O, 34.45.

*6-Carboxymethyl-5,6-dihydro-2-[(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl)methyl]-2*H*-pyran* (**4a,b,c,d**).—A solution of diene **3** (1.5 g, 6.5 mmol) and sodium glyoxylate (3.7 g, 32.5 mmol, 9:1 mixture of *E/Z* isomers) in 5 mL of water in a screw-capped tube was heated at 140 °C under vigorous stirring for 48 h. Chromatography of the solution on Dowex-1 formiate (elution with a 0–2 M aqueous formic acid gradient) followed by freeze-drying afforded the mixture of cycloadducts. A mixture of the cycloadducts and Dowex-50 (H^+) resin (1.0 g) in dry MeOH (20 mL) was stirred for 2 h at 40 °C, then filtered, and the filtrate was evaporated to dryness. The residual syrup was dissolved in pyridine (10 mL), and acetic anhydride (10 mL) was added. The mixture was stirred for 8 h at room temperature, poured in 0.5 N HCl (50 mL) and extracted with CH_2Cl_2 (3 × 80 mL). The combined organic layers were washed with a satd $NaHCO_3$ soln and brine, dried, and concentrated. The residual syrup was purified by flash chromatography (4:1 hexane–EtOAc) to afford 1.9 g (68% from the *E* isomer) of the cycloadducts **4a,b,c,d** as a 41:24:21:14 mixture (^{13}C NMR analysis). NMR (^1H, 250 MHz, $CDCl_3$): δ 1.72–2.10 (m and 4 s, 14 H, 4 $COCH_3$ and CH_2), 2.25–2.48 (m, 2 H, H-5a and H-5b), 3.76 and 3.77 (2 s, 3 H, CO_2CH_3), 3.82–4.68 (m, 6 H, H-2, H-6, H-1′, H-5′, and 2 H-6′), 4.94–5.15 (m, 2 H, H-2′ and H-4′), 5.18–5.35 (m, 1 H, H-3′), 5.62–5.94 (m, 2 H, H-3 and H-4); (^{13}C, 62.9 MHz, $CDCl_3$), selected data: δ 20.3 (4 $COCH_3$), 51.7 (CO_2CH_3), 61.7 and 61.9 (C-6′), 122.8, 123.2, 124.0, 124.2, 127.8, 128.4, 128.9, and 129.2 (C-3 and C-4). Anal. Calcd for $C_{22}H_{30}O_{12}$: C, 54.32, H, 6.23; O, 39.48. Found: C, 54.21; H, 6.49; O, 39.52.

*(2R,6S)-, (2S,6R)-, (2R,6R)-, and (2S,6S)-6-Benzoyloxymethyl-5,6-dihydro-2-[(2,3,4,6-tetra-*O*-benzoyl-α-*D*-glucopyranosyl)methyl]-2*H*-pyran* (**5**).—A mixture of **4** (1.9 g, 3.9 mmol) and $LiBH_4$ (710 mg, 25.5 mmol) in 30 mL of THF and 30 mL of *i*PrOH was stirred at room temperature for 24 h. Then MeOH (15 mL) and H_2O (15 mL) were added and the mixture was stirred for 2 h at room temperature. The solvents were evaporated and the residue was purified by flash chromatography (4:1 CH_2Cl_2–MeOH) to afford 837 mg (74%) of the four isomers. This mixture was dissolved in pyridine (4 mL), and benzoyl chloride (3 mL) was added. The mixture was stirred for 8 h at room temperature, then 5 mL of MeOH was added, and after 15 min the mixture was concentrated and the residual syrup was purified by flash chromatography (20:1 toluene–EtOAc).

Eluted first was a 40:33:27 mixture of isomers (1.2 g, 38% from **4**). NMR (^{13}C, 62.9 MHz, $CDCl_3$), selected data: δ 26.4, 26.9, 27.1, 31.8, 32.1, and 32.8 (CH_2 and C-5), 162.1, 165.2, 165.3, 165.8, and 166.1 (*C*OPh). Anal. Calcd for $C_{48}H_{42}O_{12}$: C, 71.09, H, 5.22; O, 23.69. Found: C, 70.56; H, 5.51; O, 24.06.

Eluted second was (2*S*,6*S*)-6-benzoyloxymethyl-5,6-dihydro-2-[(2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl)methyl]-2*H*-pyran (**5a**), (900 mg, 28% from **4**); $[\alpha]_D$ −4° (*c* 1.4, $CHCl_3$); IR (KBr): *ν* 2926, 1721, 1601, 1492, 1451, 1315, 1270, 1177, 1095, 1069, and 1026 cm^{-1}; NMR (^1H, 400 MHz, $CDCl_3$): δ 2.00 (ddd, 1 H, $J_{2,a}$ 13.0, $J_{1,a}$ 2.5, $J_{a,b}$ 15.0 Hz, H-a), 2.05 (m, 2 H, 2 H-5), 2.20 (ddd, 1 H, $J_{2,b}$ 3.5, $J_{1',b}$ 11.5 Hz, H-b), 3.95 (ddd, 1 H, $J_{6,7}$ 5.5, $J_{5a,6}$ 7.5, $J_{5b,6}$ 11.0 Hz, H-6), 4.29 (d, 2 H, 2 H-7), 4.34 (ddd, 1 H, $J_{5',6'a}$ 5.5, $J_{5',6'b}$ 4.0, $J_{4',5'}$ 8.5 Hz, H-5′), 4.50–4.55 (m, 2 H, H-6′a and H-2), 4.59 (dd, 1 H, $J_{6'a,6'b}$ 12.0 Hz, H-6′b), 4.85 (ddd, 1 H, $J_{1',2'}$ 5.5 Hz, H-1′), 5.57 (dd, 1 H, $J_{2',3'}$ 9.0 Hz, H-2′), 5.58 (t, 1 H, $J_{3',4'}$ 9.0 Hz, H-4′), 5.77–5.87 (m, 2 H, H-3 and H-4), 5.93 (t, 1 H, H-3′), 8.02–7.85 and 7.30–7.55 (m, 25 H, Bz); (^{13}C, 62.9 MHz, $CDCl_3$): δ 26.7, 29.9 (CH_2 and C-5); 63.3 (C-6′); 66.0, 66.5, 68.2, 69.4, 69.5, 69.8, 70.5, and 70.7 (C-1′, C-2′, C-3′, C-4′, C-5′, C-1, C-6, and C-7); 123.9, 128.3–129.8, and 132.9–133.3 (C-3, C-4, and Ph), 165.1, 165.2, 165.7, 166.1, and 166.4 (*C*OPh). Anal. Calcd for $C_{48}H_{42}O_{12}$: C, 71.09, H, 5.22; O, 23.69. Found: C, 70.61; H, 5.52; O, 23.87.

*(2S,6S)-6-Hydroxymethyl-5,6-dihydro-2-[(α-*D*-glucopyranosyl)methyl]-pyran* (**6a**).—A solution of the benzoyl derivative **5a** (800 mg, 0.98 mmol) in methanolic 0.2 M MeONa (30 mL) was kept at room temperature overnight, then neutralized with Dowex-50 (H^+) resin, filtered, and concentrated to dryness. The residue was purified by flash chromatography (4:1 CH_2Cl_2–MeOH) to afford 286 mg (100%) of

6a; $[\alpha]_D$ −15° (*c* 0.9, CH_3OH); IR (KBr): ν 3455, 2919, 1415, 1368, 1243, 1188, 1111, 1081, and 1052 cm^{-1}; NMR (^1H, 400 MHz, CD_3OD): δ 1.68 (ddd, 1 H, $J_{a,1'}$ 3.0, $J_{a,2}$ 11.5, $J_{a,b}$ 15.5 Hz, H-a), 1.80–1.90 (m, 3 H, H-b and 2 H-5), 3.11 (dd, 1 H, $J_{3',4'}$ 8.5, $J_{4',5'}$ 9.5 Hz, H-4′), 3.31 (ddd, 1 H, $J_{5',6'b}$ 2.5, $J_{5',6'a}$ 6.2 Hz, H-5′), 3.37 (dd, 1 H, $J_{2',3'}$ 9.5 Hz, H-3′), 3.43 (d, 2 H, $J_{6,7}$ 5.0 Hz, 2 H-7), 3.50 (dd, 1 H, $J_{1',2'}$ 5.7 Hz, H-2′), 3.52 (dd, 1 H, $J_{6'a,6'b}$ 12.0 Hz, H-6′a), 3.61–3.68 (m, 1 H, H-6), 3.66 (dd, 1 H, H-6′b), 4.13 (ddd, 1 H, $J_{1',b}$ 11.3 Hz, H-1′), 4.24–4.31 (m, 1 H, H-2), 5.62–5.67 and 5.70–5.76 (m, 2 H, H-3 and H-4); (^{13}C, 62.9 MHz, CD_3OD): δ 27.7 and 28.9 (C-7 and C-5); 63.2 (C-6′); 65.8, 69.4, 69.9, 72.4, 72.6, 73.1, 74.9, and 75.1 (C-1′, C-2′, C-3′, C-4′, C-5′, C-2, C-6, and C-7); 124.6 and 131.1 (C-3 and C-4). Anal. Calcd for $C_{13}H_{22}O_7 \cdot 0.3\ H_2O$: C, 52.80; H, 7.70; O, 39.5. Found: C, 52.89; H, 7.61; O, 39.8.

(2R,6S)-, (2S,6R)-, and (2R,6R)-6-Hydroxymethyl-5,6-dihydro-2-[(α-D-glucopyranosyl)methyl]-pyran (**6b,c,d**).—The mixture of isomers **5b,c,d** (900 mg, 1.1 mmol) was treated, as described for the preparation of **6a**, to obtain after flash chromatography (4:1 CH_2Cl_2–MeOH) 322 mg of **6b,c,d** as an oil (100%). NMR (^{13}C, 62.9 MHz, CD_3OD), selected data: δ 27.6, 27.9, 28.1 30.7, 31.5, and 32.2 (C-5 and C-7); 63.0, 65.8, 66.1 (C-6′); 124.7, 125.2, 130.0, 130.7, and 131.9 (C-2 and C-3). Anal. Calcd for $C_{13}H_{22}O_7$: C, 53.77; H, 7.64. Found: C, 53.15; H, 7.26.

(2S,6S)-6-Hydroxymethyl-3,4,5,6-tetrahydro-2-[(α-D-glucopyranosyl)methyl]-pyran (**7a**).—A vigorously stirred mixture of **6a** (70 mg, 0.24 mmol) and 10% palladium on activated carbon (25 mg) in MeOH (2 mL) was degassed under vacuum and saturated with hydrogen (with a H_2-filled balloon) three times. The suspension was stirred for an additional 2 h at room temperature under a slightly positive pressure of H_2 (balloon), filtered through a plug of cotton, and concentrated to afford **7a** (97 mg, ~100%) pure by NMR spectroscopy analysis. $[\alpha]_D$ +107° (*c* 0.7, CH_3OH); IR (KBr): ν 3375, 1444, 1368, 1268, 1203, 1175, 1075, and 1043 cm^{-1}; NMR (^1H, 400 MHz, CD_3OD): δ 1.30–1.51 and 1.61–1.83 (2 m, 7 H, H-b, 2 H-3, 2 H-4, 2 H-5), 2.15 (ddd, 1 H, $J_{1',a}$ 2.8, $J_{2,a}$ 11.0, $J_{a,b}$ 15.5 Hz, H-a), 3.26 (dd, 1 H, $J_{3',4'}$ 8.5, $J_{4',5'}$ 9.4 Hz, H-4′), 3.44 (ddd, 1 H, $J_{5',6'a}$ 6.3, $J_{5',6'b}$ 2.5 Hz, H-5′), 3.47 (dd, 1 H, $J_{6,7a}$ 4.5, $J_{7a,7b}$ 11.3 Hz, H-7a), 3.53 (dd, 1 H, $J_{2',3'}$ 9.3 Hz, H-3′), 3.63 (dd, 1 H, $J_{1',2'}$ 5.6 Hz, H-2′), 3.66 (dd, 1 H, $J_{6'a,6'b}$ 11.6 Hz, H-6′a), 3.69 (dd, 1 H, $J_{6,7b}$ 7.5 Hz, H-7b), 3.73–3.81 (m, 1 H, H-6), 3.81 (dd, 1 H, H-6′b), 4.01–4.07 (m, 1 H, H-2), 4.21 (ddd, 1 H, $J_{1',b}$ 12.0 Hz, H-1′); (^{13}C, 100 MHz, CD_3OD): δ 20.2, 28.3, 28.4, 31.8, 63.9, 65.3, 69.2, 73.0, 73.1, 73.4, 73.7, 75.7, and 75.7.

(2S,6S)-6-Benzoyloxymethyl-3,4,5,6-tetrahydro-2-[(2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl)methyl]-pyran (**8a**).—A solution of **7a** (10 mg, 0.24 mmol) in pyridine (200 mL) was treated with benzoyl chloride (100 μL). The mixture was stirred for 8 h at room temperature, then 200 μL of MeOH were added, and after 15 min the mixture was concentrated and the residual syrup was purified by flash chromatography (20:1 toluene–EtOAc) to afford 19 mg (95%) of **8a**. $[\alpha]_D$ +29° (*c* 1.5, $CHCl_3$); IR (KBr): ν 2934, 1722, 1602, 1451, 1316, 1270, 1094, 1069, and 1026 cm^{-1}; NMR (^1H, 400 MHz, $CDCl_3$): δ 1.00–1.40 (m, 6 H, 2 H-3, 2 H-4, 2 H-5), 1.82 (ddd, 1 H, $J_{1',b}$ 11.2, $J_{2,b}$ 3.0, $J_{a,b}$ 15.0 Hz, H-b), 2.07 (ddd, 1 H, $J_{1',a}$ 2.6, $J_{2,a}$ 10.5 Hz, H-a), 3.80 (dddd, 1 H, $J_{6,7a}$ 4.9, $J_{6,7b}$ 7.8 Hz, H-6), 3.97 (m, 1 H, H-2), 4.11 (dd, 1 H, $J_{7a,7b}$ 11.3 Hz, H-7a), 4.38 (dd, 1 H, H-7b), 4.31 (ddd, 1 H, $J_{5',6'a}$ 4.0, $J_{5',6'b}$ 5.8, $J_{4',5'}$ 8.7 Hz, H-5′), 4.57 (dd, 1 H, $J_{6'a,6'b}$ 12.0 Hz, H-6′b), 4.61 (dd, 1 H, H-6′a), 5.02 (ddd, $J_{1',2'}$ 5.6 Hz, H-1′), 5.75 (dd, $J_{3',4'}$ 8.8 Hz, H-4′), 5.78 (dd, 1 H, $J_{2',3'}$ 9.2 Hz, H-2′); 6.34 (dd, 1 H, H-3′); (^{13}C, 62.9 MHz, $CDCl_3$): δ 18.7, 26.3, 29.9, 30.5, 63.7, 64.8, 66.9, 69.2, 69.9, 70.1, 70.6, 71.3, 71.7, 127.6–133.2, 165.3, 165.6, 166.0, and 166.1. Anal. Calcd for $C_{48}H_{44}O_{12}$: C, 70.93; H, 5.46; O, 23.62. Found: C, 70.81; H, 5.51; O, 23.77.

Enzymatic tests.—The reaction mixture consisted of 130 mM citrate buffer (pH 5.7), 3 mM α,α-trehalose, porcine kidney trehalase (3.5 mU), and the potential inhibitor at different concentration (10.5, 15.8, 32.0, and 47.0 mM), for a total volume of 100 μL. The reaction mixture was incubated at 37 °C for different periods (10, 20, and 30 min) and was then quenched by heating the sample at 100 °C (boiling water bath) for 5 min. Glucose formed during the reaction was measured by the glucose hexokinase–glucose-6-phosphate dehydrogenase method using the commercially available glucose (HK) kit (Sigma).

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