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ASYMMETRIC ACETALATION OF α,α-TREHALOSE: SYNTHESIS OF α-D-GALACTOPYRANOSYL α-D-GLUCOPYRANOSIDE AND 6-DEOXY-6-FLUORO-α-D-GLUCOPYRANOSYL α-D-GLUCOPYRANOSIDE*

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ABSTRACT

Selective acetalation of α,α-trehalose with ethyl or methyl isopropenyl ether and toluene-*p*-sulphonic acid in *N*,*N*-dimethylformamide gave the 4,6-isopropylidene acetal as the major product, isolated as its hexa-acetate **1** (38%). The *gluco-galacto* analogue **6** of α,α-trehalose was synthesized from **1** by the sequence: hydrolysis of the isopropylidene group with trifluoroacetic acid, mesylation of the resulting diol, benzoate displacement, and saponification of the product. Deacetylation of **1** followed by benzylation and hydrolysis of the acetal group furnished a hexa-*O*-benzyl derivative **9**. Tosylation of the primary hydroxyl group in **9**, treatment of the product with tetrabutylammonium fluoride in acetonitrile, and subsequent catalytic hydrogenolysis of the benzyl groups gave 6-deoxy-6-fluoro-α,α-trehalose (**12**). Compounds **6** and **12** and 6-deoxy-6-iodo-α,α-trehalose are substrates for cockchafer trehalase, but have very low V_{max} values.

INTRODUCTION

Asymmetric derivatives of the symmetrical disaccharide α,α-trehalose (α-D-glucopyranosyl α-D-glucopyranoside) are of interest for the study of the mechanism of action of trehalases (α,α-trehalose glucohydrolases, EC 3.2.1.28), which are a

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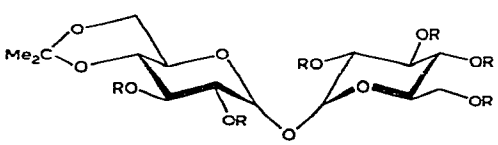
widespread group of highly specific glycosidases. They may also be useful as bactericides[2], fungicides, or insecticides, and simple methods of synthesis are therefore important. α,α-Trehalose is an essential link in the transport of D-glucose in insects[3]. Furthermore, trehalases are present in higher mammals[4], where they may also play an essential role in the transport of D-glucose[5].

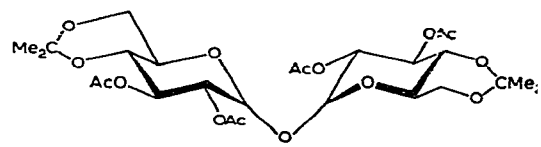
Substitution at one of two equivalent sites in α,α-trehalose has been achieved by stoichiometric esterification[1,6,7], by controlled halogenation[8] with triphenylphosphine–*N*-bromosuccinimide, or by selective hydrolysis of a symmetrical dibenzylidene acetal[9]. However, all the reported examples have afforded mixtures, and their utility is largely dependent upon the proportion of monosubstituted derivatives formed. The acetonation procedure[10] that affords kinetic products by use of alkyl isopropenyl ethers provides convenient access to the 4,6-monoisopropylidene acetal of α,α-trehalose, and we now report on its use.

RESULTS AND DISCUSSION

Optimal conditions for obtaining the maximum proportion of monoacetal by the reaction of α,α-trehalose with ethyl or methyl isopropenyl ether, below 5° in *N,N*-dimethylformamide containing a catalytic amount of toluene-*p*-sulphonic acid, were determined by monitoring reactions by g.l.c. of the trimethylsilylated product mixtures. Although 4–5 mol. of the reagent were necessary for complete reaction of



1 R = Ac
8 R = Bzl



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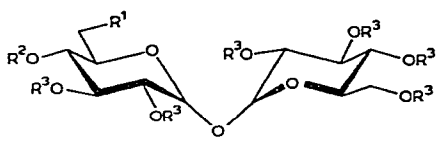
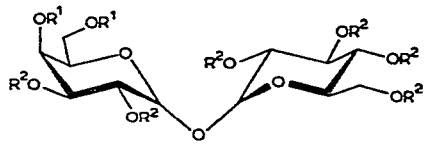
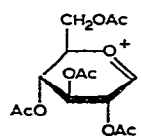


3 R^1 = OH, R^2 = H, R^3 = Ac
4 R^1 = OMs, R^2 = Ms, R^3 = Ac
9 R^1 = OH, R^2 = H, R^3 = Bzl
10 R^1 = OTs, R^2 = H, R^3 = Bzl
11 R^1 = F, R^2 = H, R^3 = Bzl
12 R^1 = F, R^2 = R^3 = H
13 R^1 = F, R^2 = R^3 = Ac
14 R^1 = I, R^2 = R^3 = H



5 R^1 = Bz, R^2 = Ac
6 R^1 = R^2 = H
7 R^1 = R^2 = Ac



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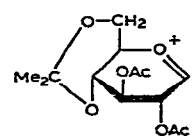

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all of the α,α-trehalose, the highest yield of monoacetal was obtained by using 1.5–1.8 mol. The desired product was isolated most conveniently by acetylation of the crude mixture, followed by separation of 4,6-*O*-isopropylidene-α-D-glucopyranosyl α-D-glucopyranoside hexa-acetate (**1**, 38%) from α,α-trehalose octa-acetate and the tetra-acetate **2** of the symmetrical diacetal.

The mass spectrum of **1** showed signals at *m/e* 331 and 287, corresponding to the formation of the two expected glycosyl carbonium ions **15** and **16**. The n.m.r. spectrum of **1** showed a triplet at low field (δ 5.06, *J* 10 Hz) attributed to H-4 of the non-acetonated D-glucosyl residue, together with the anticipated ABX set of signals at higher field that is characteristic of H-5,6,6′ of acetylated glycopyranosides. The signals for the corresponding protons of the acetonated residue appeared, as expected, at higher field, and were not readily differentiated.

Deacetonation of **1** by aqueous trifluoroacetic acid at 20° gave a high yield of 2,3,4,6,2′,3′-hexa-*O*-acetyl-α,α-trehalose (**3**), which has also been obtained[11] by hydrolysis of the 4,6-*O*-benzylidene analogue of **1**. Conversion of **3** into the corresponding 4′,6′-di-*O*-mesyl derivative **4**, followed by treatment with sodium benzoate in hexamethylphosphoric triamide, led to 2,3-di-*O*-acetyl-4,6-di-*O*-benzoyl-α-*D*-galactopyranosyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**5**). The ^{1}H-n.m.r. spectrum of **5** showed the anticipated signal for H-4 of a galactopyranosyl residue at δ 5.93 with $J_{3,4}$ 3.5 and $J_{4,5}$ 1.5 Hz. Zemplén deacylation of **5** gave α-D-galactopyranosyl α-D-glucopyranoside (**6**), which has also been obtained[11] *via* 4,6-*O*-benzylidene-α,α-trehalose, and from which a crystalline octa-acetate **7** was obtained.

6-Deoxy-6-fluoro-α,α-trehalose (**12**) was synthesized *via* 2,3,4,6,2′,3′-hexa-*O*-benzyl-α,α-trehalose (**9**). An attempt to obtain **9** by benzylation of the crude acetonation product from α,α-trehalose, with subsequent deacetonation, failed because the chromatographic mobility of the monoacetal hexabenzyl ether differed very little from that of the diacetal tetrabenzyl ether. It was thus necessary to prepare **9** by benzylation of pure 4,6-*O*-isopropylidene-α,α-trehalose obtained by Zemplén deacetylation of **1**. An alternative procedure, involving benzylation of **1**, gave a lower overall yield of **9**. Reaction of **9** with a slight stoichiometric excess of toluene-*p*-sulphonyl chloride in pyridine gave the 6-sulphonate **10** in almost quantitative yield. Subsequent treatment of **10** with tetrabutylammonium fluoride gave 48% of the fluoro derivative **11**, together with 35% of **9** arising by sulphonic ester cleavage. A similar side-reaction was encountered[12] in the conversion of 2,3,2′,3′-tetra-*O*-benzyl-4,6,4′,6′-tetra-*O*-mesyl-α,α-trehalose into the 6,6′-difluoro derivative.

Hydrogenolysis of **11** afforded a quantitative yield of 6-deoxy-6-fluoro-α,α-trehalose (**12**), which was characterized as the crystalline hepta-acetate **13**. The ^{19}F-n.m.r. spectrum of **13** showed a second-order multiplet at 75 p.p.m., with $J_{6,F} + J_{6',F}$ 92.5 Hz and $J_{5,F}$ 21.5 Hz confirming the presence of a $-CH_2F$ grouping.

The disaccharides **6** and **12** were tested as substrates or potential inhibitors of cockchafer trehalase[1,13] of specific activity 307. The K_m of the enzyme (0.617 mM for α,α-trehalose) is only slightly altered in the 6-fluoro derivative **12** (0.645 mM), but markedly increased (3.33 mM) for the galactosyl glucoside **6**. The V_{max} values for

6 and **12** were 15 and 7.5%, respectively, of the V_{max} value for trehalose. 6-Deoxy-6-iodo-α,α-trehalose (**14**) has K_m 200 mM and V_{max} 3.7%; at low concentrations, this compound acted as a competitive inhibitor with respect to α,α-trehalose (K_i 0.2 mM).

EXPERIMENTAL

General methods. — Solutions were dried over Na_2SO_4 and concentrated *in vacuo* at <45°. T.l.c. was performed on silica gel F-254 (Merck) with *1*, ethyl acetate–light petroleum (1:1); *2*, ethyl acetate–hexane (3:1); *3*, ethyl acetate–hexane (1:1); *4*, ether–hexane (4:1); *5*, ether–hexane (2:1); *6*, ether–hexane (1:1); and *7*, ethyl acetate–methanol (2:1); or, for **13**, on cellulose (plastic sheets F 1440 LS 254) with *8*, 1-butanol–pyridine–water–acetic acid (6:4:3:1). Column chromatography was performed on silica gel 60 (Merck, 70–230 mesh). Whatman No. 1 paper was used for descending chromatography of **6** with *9*, 1-butanol–pyridine–water (6:4:3). G.l.c. was performed on a Hewlett Packard model 5270 apparatus (flame-ionization detector) with a nitrogen flow-rate of 30 ml/min, and a glass column (3 mm × 2 m) packed with 2% of OV-1 maintained at 220°; the extremity was also packed and fitted into the injector (250°) to permit direct injection onto the column. Retention times (T_R) are given relative to that of trimethylsilylated α-D-glucose and in seconds (in parentheses). Optical rotations were determined with a Quick Polarimeter (Roussel et Jouan) or with a Perkin–Elmer model 141 spectropolarimeter at room temperature. ^{1}H-N.m.r. spectra (250 MHz, Table I) were recorded by M. Reutenauer at the Laboratoire Grenoblois de Resonance Magnétique Nucléaire, with a Cameca spectrometer operating in the frequency sweep-mode, with 5% of Me_4Si as the lock signal and internal reference; chemical shifts are reported as δ values (p.p.m.). Assignments were usually confirmed by the INDOR technique. The ^{19}F-n.m.r. spectrum of **13** was recorded with a Brucker WP-60 instrument at 56.4 MHz, with hexafluorobenzene as internal reference. Mass spectra (70 eV) were determined by M. C. Bosso, with an A.E.I. MS-30 instrument, coupled with a Varian 100 MS data-acquisition processing system, at an accelerating voltage of 2 kV and an electronic current at 100 μA. The temperature of the source was usually between 150 and 250°. Relative intensities against the base peak are given in parentheses.

2,3-Di-O-acetyl-4,6-O-isopropylidene-α-D-glucopyranosyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (**1**) *and 2,3-di-O-acetyl-4,6-O-isopropylidene-α-D-glucopyranosyl 2,3-di-O-acetyl-4,6-O-isopropylidene-α-D-glucopyranoside* (**2**). — To a stirred mixture of anhydrous α,α-trehalose[14] (3.42 g, 10 mmol), dry *N,N*-dimethylformamide (40 ml) below 5°, and Sikkon (Fluka dehydrating agent, 1 g) were added ethyl isopropenyl ether (1.29 g, 15 mmol) [or methyl isopropenyl ether (1.08 g, 15 mmol)] and toluene-*p*-sulphonic acid (~20 mg). The mixture was stirred for 4 h at 0–5°, sodium carbonate (~2 g) was added, and the mixture was stirred vigorously for 1 h, filtered, and concentrated at 40°/<1 Torr.

To a solution of the residue (4.9 g) in pyridine (5 ml) was added a solution of acetic anhydride (15 ml) in pyridine (15 ml) with stirring at 0°. The mixture was

TABLE I

N.M.R. DATA[a]

Compound	1	2	3	4	5	7	13
H-1	5.22 d[b]	5.25 d	5.25 d	5.31 d	5.34 d	~5.35	5.29 d[b]
H-1′	5.35 d[b]	5.25 d	5.33 d	5.34 d	5.51	5.33	5.30 d[b]
H-2	4.98 dd[b]	4.92 dd	4.99 dd	5.08 dd	5.43 dd	5.31 dd	5.02 dd
H-2′	4.99 dd[b]	4.92 dd	4.99 dd	5.02 dd	5.12 dd	5.08 dd	≃5.05
H-3	5.51 t	5.43 t	5.38 t	5.50 t	5.54 dd	5.4 dd	5.50 t
H-3′	5.44 t	5.43 t	5.50 t	5.60 t	5.50 t	5.52 t	5.52 t
H-4	3.76 m	3.78 m	3.82 m	4.73 t	5.93 dd	5.56 m	5.07 t
H-4′	5.06 t	3.78 m	5.07 t	5.08 m	5.06 t	5.06 t	5.05 t
H-5	3.76 m	3.78 m	3.70 m	4.0 m	4.60 t	4.36 m	4.19 m
H-5′	4.08 o	3.78 m	4.12 o	4.10 m	4.10 m	~4.20 m	4.11 o
H-6	3.76 m	3.78 m	3.82 m 3.92 m	4.0 m	4.46 m 4.00 m	~4.20 m	4.40 md
H-6′	4.28 dd 4.01 dd	3.78 m	4.25 dd 4.01 dd	4.44 dd 4.35 dd	4.22 m 4.05 m	~4.20 m	4.27 dd 4.0 dd
$J_{1,2}$	4.0	4.0	4.0	4.0	3.5	—	3.5
$J_{1',2'}$	4.0	4.0	3.5	3.5	4.5	4.0	4.0
$J_{2,3}$	10.0	10.0	10.0	10.0	10.5	10.5	10.0
$J_{2',3'}$	10.0	10.0	10.0	10.0	10.0	10.0	—
$J_{3,4}$	10.0	10.0	10.0	10.0	3.5	⩾4	10.0
$J_{3',4'}$	10.0	10.0	10.0	10.0	10.0	10.0	9.5
$J_{4,5}$	—	—	10.0	—	1.5	⩾4	10.0
$J_{4',5'}$	10.0	—	10.0	10.0	10.0	10.0	9.5
$J_{5,6}$	—	—	—	—	—	—	—
$J_{5',6'}$	4.5,3	—	5.0,2.5	6.0,3.0		—	5.0,2.0
$J_{6a,6b}$	—	—	—	—	—	—	—
$J_{6'a,6'b}$	12.0	—	12.0	11.0		—	12.0

[a]First-order chemical shifts (δ). Key: s, singlet; d, doublet; dd, double doublet; o, octet; t, triplet; m, multiplet; md, multiplet of doublets. The primed numbers refer to the glycopyranosyl ring that is more highly acetylated. Spin couplings are given in Hz. [b]The primed and non-primed protons are not differentiated.

stirred overnight at room temperature and then poured onto ice. The product was extracted with dichloromethane, and the extract was concentrated to give an amorphous solid (5.6 g). T.l.c. (solvent *1*) revealed three products, R_F 0.69, 0.56, and 0.42. Elution (solvent *1*) from silica gel (400 g) gave, first, **2** (0.8 g), m.p. 187–189°, $[\alpha]_D$ +142° (*c* 1, chloroform), +148° (*c* 1.2, acetone), R_F 0.69.

Anal. Calc. for $C_{26}H_{38}O_{15}$: C, 52.88; H, 6.44; O, 40.68. Found: C, 52.84; H, 6.42; O, 40.80.

Eluted second was **1** (2.4 g, 38%), m.p. 79–80°, $[\alpha]_D$ +150.5° (*c* 1.1, chloroform), and R_F 0.56.

Eluted third was octa-*O*-acetyl-α,α-trehalose (1.0 g), m.p. 97–98°, $[\alpha]_D$ +157° (*c* 1.1, chloroform), R_F 0.42; lit.[15] m.p. 98°, $[\alpha]_D$ +162° (chloroform).

The foregoing, optimal conditions for monoacetonation were determined by monitoring reactions. Aliquot portions were neutralized with sodium carbonate, and

a portion (1 ml) was treated at ~50° with *N*-trimethylsilylimidazole (1 ml) in a closed vial for 15 min. In g.l.c., the trimethylsilylated components of the product mixture had the following T_R values: **2**, 9.52 (1495 sec); **1**, 11.59 (1820 sec); trehalose, 13.15 (2065 sec).

2,3-Di-O-acetyl-α-D-glucopyranosyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (**3**). — A solution of **1** (2 g) in 9:1 trifluoroacetic acid–water (10 ml) was stirred for 5 min at room temperature and then concentrated. Several portions of toluene were evaporated *in vacuo* from the residue, which was then eluted from silica gel (100 g) with ethyl acetate to give **3** as an amorphous solid (1.89 g, 93%), m.p. 110–112°, $[\alpha]_D$ +134° (*c* 0.75, chloroform); lit.[11] m.p. 106–108° (from ethanol).

Anal. Calc. for $C_{24}H_{34}O_{17}\cdot H_2O$: C, 47.06; H, 5.92. Found: C, 47.28; H, 5.66.

After drying for 24 h at 70° *in vacuo*, the product had m.p. 113–114°, $[\alpha]_D$ +140° (*c* 1, chloroform).

Anal. Calc. for $C_{24}H_{34}O_{17}$: C, 48.48; H, 5.76. Found: C, 48.62; H, 5.95.

2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl 2,3-di-O-acetyl-4,6-di-O-methanesulphonyl-α-D-glucopyranoside (**4**). — To a solution of **3** (1.6 g) in pyridine (10 ml) at ~0° was added methanesulphonyl chloride (1 ml). The mixture was left overnight at 0° and then poured into ice–water (100 ml). The precipitate was collected, washed with cold water, and dried. The product (2 g) was homogeneous in t.l.c. (solvent *2*). Elution (solvent *2*) of a portion from silica gel gave **4** as an amorphous solid, m.p. 84–86°, $[\alpha]_D$ +110° (*c* 1, chloroform); lit.[11] m.p. 93–95° (from 2-propanol), $[\alpha]_D$ +102° (*c* 0.45, chloroform).

Anal. Calc. for $C_{23}H_{38}O_{21}S_2$: C, 41.60; H, 5.10; S, 8.54. Found: C, 41.56; H, 5.19; S, 8.68.

2,3-Di-O-acetyl-4,6-di-O-benzoyl-α-D-galactopyranosyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (**5**). — A mixture of **4** (823 mg, 1.09 mmol), sodium benzoate (823 mg), and hexamethylphosphoric triamide (8 ml) was heated for 20 h at 100°. The cooled mixture was poured into ice–water (100 ml), and the precipitate (865 mg, 98%) was treated as in the preceding experiment and purified by column chromatography (solvent *4*) to give **5** as a foam (635 mg, 73%), m.p. 78–83°, $[\alpha]_D$ +125° (*c* 1, chloroform).

Anal. Calc. for $C_{38}H_{42}O_{19}$: C, 56.85; H, 5.27. Found: C, 56.29; H, 5.25.

α-D-Galactopyranosyl α-D-glucopyranoside (**6**). — To a solution of **5** (600 mg) in methanol (20 ml) was added a small piece of sodium. The solution was kept at room temperature for 20 h and then concentrated to give **6** as a foam that was homogeneous by p.c. (solvent *9*); R_{Glc} 0.51, $[\alpha]_D$ + 208° (*c* 0.8, water); lit.[11] $[\alpha]_D$ +183° (*c* 0.6, water).

Anal. Calc. for $C_{12}H_{22}O_{11}$: C, 42.10; H, 6.47. Found: C, 41.93; H, 6.27.

Hydrolysis of **6** (5 mg) with 2M hydrochloric acid (1 ml) for 1 h at 100° gave equal amounts of galactose and glucose (p.c., solvent *9*).

Conventional treatment of **6** with acetic anhydride–pyridine, followed by elution (solvent *3*) of the crude product from silica gel, gave the octa-acetate **7**, m.p. 76–78°, $[\alpha]_D$ +196° (*c* 0.25, chloroform).

Anal. Calc. for $C_{28}H_{38}O_{19}$: C, 49.55; H, 5.64. Found: C, 49.73; H, 5.78.

2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl 2,3-di-O-benzyl-α-D-glucopyranoside (**9**). — Zemplén deacetylation of **1** (3.95 g, 6.2 mmol) in methanol (30 ml) gave, after 2 h, a quantitative yield of mono-*O*-isopropylidenetrehalose (2.45 g). The product, which was homogeneous in t.l.c. (solvent *7*), was treated in methyl sulphoxide (65 ml) with powdered potassium hydroxide (40 g) and benzyl bromide (12 ml) for 2 h at room temperature. After addition of diethyl ether (100 ml), the mixture was filtered, and washed with water (3 × 100 ml). The washings were extracted with ether (3 × 100 ml), and the combined organic phases were concentrated. The oily residue was eluted from a column (35 × 4 cm) of silica gel (150 g); hexane (1 litre) followed by ether (1 litre) gave **8** (5.9 g) which was almost pure by t.l.c. (solvent *5*). This product was treated with 1 : 1 methanol–90% acetic acid (160 ml) for 24 h at room temperature. Concentration of the solution (with the addition of portions of toluene) and elution (solvent *4*) of the residue from silica gel gave **9** (4.3 g, 76%), $[\alpha]_D$ +90° (*c* 3.09, chloroform); *m/e*: 415 (0.8, glycosyl$^+$ −OBzl), 343 (0.7, 2,3-di-*O*-benzylglycosyl$^+$), 325 (2.0, 343 − H_2O), and 217 (1.9, benzyloxypyranyl$^+$).

Anal. Calc. for $C_{54}H_{58}O_{11}$: C, 73.45; H, 6.62. Found: C, 73.59; H, 6.48.

Treatment of **1** (500 mg) in methyl sulphoxide (10 ml) with powdered potassium hydroxide (5 g) and benzyl bromide (1 ml) for 3 h gave **9** after similar isolation and purification on a column (solvent *5*), but only in 47% yield.

2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl 2,3-di-O-benzyl-6-O-toluene-p-sulphonyl-α-D-glucopyranoside (**10**). — A solution of **9** (1.1 g, 1.24 mmol) in pyridine (10 ml) was treated with toluene-*p*-sulphonyl chloride (355 mg, 1.87 mmol) for 24 h at room temperature. The mixture was washed with water (3 × 50 ml) and extracted with dichloromethane (3 × 50 ml), and the extract was concentrated. Elution (solvent *6*) of the product from silica gel gave **10** (1.2 g, 97%), $[\alpha]_D$ +75° (*c* 0.9, chloroform); *m/e*: 523 (0.15, tetra-*O*-benzylglycosyl$^+$), 325 (1.1, di-*O*-benzyl-6-deoxy-5-eno-glycosyl$^+$), 217 (2.1, 325 − $PhCH_2OH$).

Anal. Calc. for $C_{61}H_{64}O_{13}S$: C, 70.64; H, 6.22; S, 3.09. Found: C, 70.40; H, 6.11; S, 3.55.

2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl 2,3-di-O-benzyl-6-deoxy-6-fluoro-α-D-glucopyranoside (**11**). — A solution of **10** (1.65 g, 1.58 mmol) in acetonitrile (5 ml) was added to a stirred solution of tetrabutylammonium fluoride (4.4 g) in acetonitrile (5 ml). The reaction was monitored by t.l.c. After 8 h, the mixture was diluted with dichloromethane (100 ml) and washed with ice–water (2 × 100 ml), and the aqueous phase was extracted with dichloromethane (2 × 100 ml). The organic solutions were combined and concentrated, and the residue was eluted (solvent *6*) from silica gel (60 g) to give, first, **11** as a syrup (680 mg, 48%), $[\alpha]_D$ +96° (*c* 2.8, chloroform); *m/e*: 345 (0.7, 2,3-di-*O*-benzyl-6-deoxy-6-fluoroglycosyl$^+$), and 219 (3.0, 2-(fluoro-methyl)benzyloxypyranyl$^+$).

Anal. Calc. for $C_{54}H_{57}FO_{10}$: C, 73.28; H, 6.49; F, 2.15. Found: C, 72.86; H, 6.50; F, 2.70.

Eluted second was **9** (500 mg, 35%).

6-Deoxy-6-fluoro-α-D-glucopyranosyl α-D-glucopyranoside (**12**). — A solution of **11** (550 mg) in ethanol (20 ml) was hydrogenated over 10% palladium-on-charcoal (550 mg) at 5 atmos. for 24 h. The mixture was filtered through Celite and concentrated to give **12** (220 mg), $[\alpha]_D$ +170° (*c* 0.78, water), $R_{Trehalose}$ 2.13 (solvent *8*).

Anal. Calc. for $C_{12}H_{21}FO_{10}$: C, 41.86; H, 6.15; F, 5.52. Found: C, 42.28; H, 6.07; F, 4.92.

The hepta-acetate **13** of **12** had m.p. 82–83° (from ether), $[\alpha]_D$ +166° (*c* 0.65, chloroform).

Anal. Calc. for $C_{26}H_{35}FO_{17}$: C, 48.90; H, 5.52; F, 2.97. Found: C, 48.95; H, 5.50; F, 2.67.

6-Deoxy-6-iodo-α-D-glucopyranosyl α-D-glucopyranoside (**14**). — A solution of hepta-*O*-acetyl-6-deoxy-6-iodo-α,α-trehalose[7] (500 mg) in 90% aqueous methanol (50 ml) was treated with triethylamine (1 ml) for 12 h at room temperature, neutralized with Amberlite IR-120 (H^+) resin, and lyophilized to give **14** as a solid foam (300 mg, 80%), $[\alpha]_D$ +133° (*c* 1, water).

Anal. Calc. for $C_{12}H_{21}IO_{10}$: C, 31.87; H, 4.68; I, 28.06. Found: C, 31.68; H, 4.98; I, 26.06.

Enzyme experiments. — Cockchafer trehalase, prepared as previously described[1], was further purified by passage through a column of DEAE-cellulose. The reaction medium consisted of McIlvaine buffer (20 mM, pH 6.25), enzymic protein (0.07 μg/ml), and various dilutions of **6**, **12**, and **14**: 10, 5, 2.5, 1.25, and 0.625 mM for trehalose; 20, 10, 5, 2.5, and 1.25 mM for **6**; 20, 10, 5, and 2.5 mM for **12**; 200, 100, 50, and 25 mM for **14**. The incubation time was 30 min at 37° for α,α-trehalose and **6**; 120 and 300 min for **12**; and 250 min for **14**.

The inhibition assays were performed at two different concentrations (4 and 10 mM) of **14**, with the same concentration of trehalose as for the standard assay for trehalase activity. The hydrolysis was monitored after 0, 15, 30, and 60 min, and 6 h by t.l.c. (silica gel, propanol–ethanol–ethyl acetate–acetic acid–pyridine–water, 7:3:3:2:2:3).

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