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SYNTHESIS OF THE TETRASACCHARIDE CORE REGION OF ANTIGENIC LIPO-OLIGOSACCHARIDES CHARACTERISTIC OF *Mycobacterium kansasii**

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ABSTRACT

The oligosaccharide core region, β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1↔1)-α-D-Glc*p* (**1**), of the lipo-oligosaccharide-type antigens isolated from *M. kansasii* has been synthesised from 2,3,2′,3′,4′,6′-hexa-*O*-benzyl-6-*O*-(1-phenylethyl)-α,α-trehalose (**4**). Compound **4** was obtained by $LiAlH_4$–$AlCl_3$-type hydrogenolysis of 2,3,2′,3′,4′,6′-hexa-*O*-benzyl-4,6-*O*-(*S*)-(1-phenylethylidene)-α,α-trehalose. The β-laminaribiosyl part of the molecule was built-up by sequential glycosylation steps using 2,4,6-tri-*O*-acetyl-3-*O*-allyl-α-D-glucopyranosyl bromide in the presence of $HgBr_2$ and methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside promoted by methyl triflate. The complete *a priori* ^{13}C-n.m.r. spectrum assignment of **1** was achieved by applying 2D methods.

INTRODUCTION

Mycobacterium kansasii is characterised by the presence of seven species-specific neutral lipo-oligosaccharide antigens[1] which have, in common, a tetrasaccharide core with the following structure: β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1↔1)-α-D-Glc*p*. In the antigens, residues of xylose, 3-*O*-methylrhamnose, fucose, and *N*-acylkansosamine [4,6-dideoxy-3-*C*-methyl-2-*O*-methyl-4-(2-methoxypropionamido)-L-*manno*-hexose] are linked variously to the core, and the resulting higher oligosaccharides are substituted with 2,4-dimethyltetradecanoyl and acetyl groups[2]. The clinical problems associated with *M. kansasii* are chronic, persistent pulmonary infections resembling classical tuberculosis.

Trehalose derivatives are especially widespread within mycobacteria[3,4], but some other bacteria also contain substituted trehalose, including glycosylated trehaloses. Thus, 3-*O*-α-D-glucopyranosyl-α,β-trehalose[5] was isolated from *Strepto-*

*Dedicated to Professor Reszö Bognár in the year of his 75th birthday.

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coccus faecalis, 2-*O*-α-D-glucopyranosyl-α,α-trehalose[3] was found in *Selaginella kraussinana*, and recently the isolation of a tetra- [β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→6)-α-D-Glc*p*-(1↔1)-α-D-Glc*p*] and a penta-saccharide [3-*O*-Me-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→6)-α-D-Glc*p*-(1↔1)-α-D-Glc*p*] from *M. smegmatis*[6] was reported. The last two oligosaccharides have been synthesised in our laboratory[7].

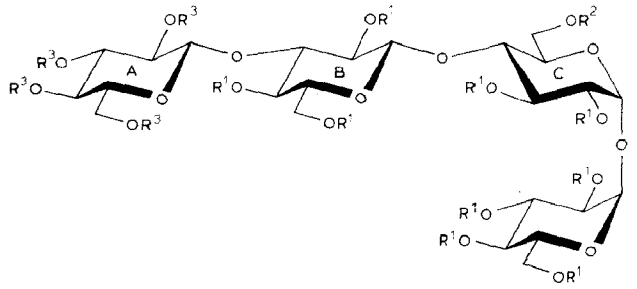
In order to avoid the difficulties emerging during the synthesis[8] of trehalose-type oligosaccharides, we started from trehalose, but encountered the problems of the synthesis of non-symmetrical, partially protected trehaloses. These were overcome by the preparation of 2,3,2′,3′,4′,6′-hexa-*O*-benzyl-6-*O*-(1-phenylethyl)-α,α-trehalose (**4**), which was used as the aglycon.

RESULTS AND DISCUSSION

For the preparation of **1**, a 2 + 2 block synthesis might seem to be suitable, but our earlier experiences concerning the reactivity of acylhalogeno laminaribiose derivatives[7] discouraged us from following this route. Hence, a step-by-step synthesis of **1** was chosen and **4** was used as glycosyl acceptor.

The starting material, 2,3,2′,3′,4′,6′-hexa-*O*-benzyl-α,α-trehalose, was obtained by a modification[9] of the original synthesis[10] and it was converted into 2,3,2′,3′,4′,6′-hexa-*O*-benzyl-4,6-*O*-(*S*)-(1-phenylethylidene)-α,α-trehalose (**2**). Hexopyranosides react with acetophenone dimethyl acetal to give diastereoisomeric 4,6-(1-phenylethylidene) acetals[11]. In the kinetic products, the methyl group is axial, but they rearrange completely into axial-phenyl isomers, the thermodynamic products. Hydrogenolysis of the latter products yields, with excellent stereo- and regio-selectivity, the 6-(*S*)-(1-phenylethyl) ethers. This method is an alternative route to the hydrogenolysis[12–14] of benzylidene acetals, using sodium cyanoborohydride–acids or Lewis acids, to prepare hexopyranosides with HO-4 free.

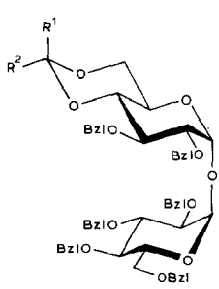
On treatment of 2,3,2′,3′,4′,6′-hexa-*O*-benzyl-α,α-trehalose[9,10] with acetophenone dimethyl acetal in the presence of toluene-*p*-sulphonic acid as



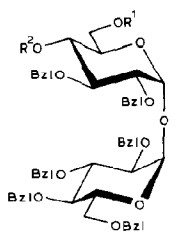
1 $R^1 = R^2 = R^3 = H$

10 $R^1 = Bzl$, $R^2 = CH(Me)(Ph)$, $R^3 = Ac$

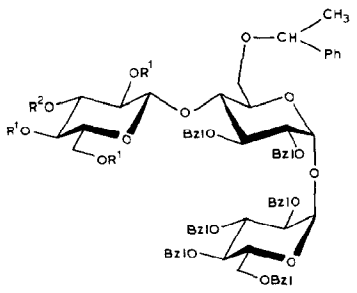
11 $R^1 = R^2 = R^3 = Ac$



2 R^1 = Ph, R^2 = Me
3 R^1 = H, R^2 = Ph



4 R^1 = CH(Ph)Me, R^2 = H
5 R^1 = H, R^2 = Bzl



6 R^1 = R^2 = Ac
7 R^1 = H, R^2 = $-CH_2-CH=CH_2$
8 R^1 = Bzl, R^2 = $-CH_2-CH=CH_2$
9 R^1 = Bzl, R^2 = H

catalyst but without any solvent, a very fast reaction took place and only the thermodynamic product (**2**) was formed. The postulated axial-phenyl structure of the dioxane-type acetal was based on the chemical shift of the resonance of the methyl carbon (30.41 p.p.m.) indicating an equatorial location. For an axial position, the resonance should appear at ~21–22 p.p.m. Hydrogenolysis of **2** with the $LiAlH_4$–$AlCl_3$ reagent gave 2,3,2′,3′,4′,6′-hexa-*O*-benzyl-6-*O*-(1-phenylethyl)-α,α-trehalose (**4**) in excellent yield. The regioselectivity of the ring-cleavage was proved by the chemical shift of the C-6 resonance (66.62 p.p.m.) and the high stereoselectivity of the reduction was demonstrated by the presence of a sharp doublet (1.42 p.p.m.) in the ^{1}H-n.m.r. spectrum. Previous investigations[11] showed that hydrogenolysis of 4,6-*O*-(1-phenylethylidene) derivatives yielded (*S*)-(1-phenylethyl) ethers. Thus, it might be assumed that the new chiral center in **4** was also *S*.

Brief treatment of 2,3,2′,3′,4′,6′-hexa-*O*-benzyl-α,α-trehalose[9,10] with α,α-dimethoxytoluene gave 2,3,2′,3′,4′,6′-hexa-*O*-benzyl-4,6-*O*-benzylidene-α,α-trehalose (**3**), hydrogenolysis of which gave 2,3,4,2′,3′,4′,6′-hepta-*O*-benzyl-α,α-trehalose (**5**). A previous synthesis of **5** was achieved[10] using conventional tritylation, benzylation, and detritylation steps.

The reaction of **4** with acetobromoglucose in the presence of $HgBr_2$ gave 65% of **6**; no trace of the α anomer could be detected. The ^{13}C-n.m.r. spectrum of **6** accorded with the structure assigned. Thus, C-1 of the newly formed β-D-glucopyranosyl unit resonated at 100.60 p.p.m., the other two anomeric carbons at 94.46 and 94.75 p.p.m., and the glucosylated C-4 of trehalose at 77.36 p.p.m.

Previous experience has shown the low reactivity of acetobromolaminaribiose under Koenigs–Knorr conditions and, therefore, instead of a 2 + 2 block synthesis, a step-by-step procedure was performed. Compound **4** was glycosylated with 2,4,6-tri-*O*-acetyl-3-*O*-allyl-α-D-glucopyranosyl bromide[15] and the products were saponified to give 66% of the trisaccharide derivative **7**. Conventional benzylation of **7** gave **8**, which was *O*-deallylated[16,17] using tris(triphenylphosphine)rhodium(I) chloride as catalyst, to yield 70% of **9** having HO-3″ free. Pilot experiments showed

that the best results for the preparation of the tetrasaccharide could be obtained by using the methyl triflate-promoted glycosylation procedure[18,19]. Thus, **9** was treated with methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside in the presence of 4 equiv. of methyl triflate to give 71.6% of the tetrasaccharide derivative **10**. The β configuration of the newly formed glucopyranosyl unit was verified by the chemical shift of the resonance of C-1‴ (101.96 p.p.m.). Catalytic hydrogenolysis of **10** and acetylation of the product gave the crystalline tetradeca-acetate **11**. The ^{13}C-n.m.r. spectrum of **11** contained only two signals for anomeric carbons because those of C-1 and C-1′ had the same chemical shifts (91.43 p.p.m.), as did those of C-1″ and C-1‴ (101.06 p.p.m.). From the four C-6 lines, two (C-6 and C-6′) appeared with the same value (61.94 p.p.m.) but with double intensities. The tetrasaccharide structure was clearly demonstrated by two low-field signals at 78.14 (C-3″) and 76.57 p.p.m. (C-4′). Zemplén deacetylation of **11** gave amorphous **1**,

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFT DATA FOR THE PROTECTED INTERMEDIATES

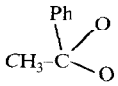
Carbon atoms	**1**	**2**	**3**	**4**	**6**	**10**	**11**
1	94.28	94.62	94.86	94.81	94.75	94.79	91.43
2	71.58	78.96	79.04	79.08	79.11	78.83	70.03
3	73.40	81.73	81.77	81.72	81.58	81.84	70.03
4	70.53	77.85	77.89	77.91	77.83	77.68	68.26
5	72.91	70.72	70.75	70.70	70.66	70.57	68.00
6	61.44	68.42	68.48	68.42	68.30	67.21	61.94
1′	93.93	93.86	94.02	93.65	94.46	94.69	91.43
2′	71.54	79.63	79.67	80.20	79.86	80.28	69.86
3′	71.90	79.20	79.04	81.10	79.96	81.18	69.36
4′	79.71	75.93	79.77	71.42	77.36	76.43	76.57
5′	69.30	63.75	62.15	69.95	70.66	70.48	66.96
6′	60.83	63.81	68.92	66.81	66.67	66.62	61.94
1″	102.86				100.60	100.30	101.06
2″	73.53				71.50	78.83	71.10
3″	85.22				73.27	83.11	78.14
4″	68.92				68.22	78.83	67.53
5″	76.30				71.91	69.63	72.13
6″	61.48				61.67	67.21	62.14
1‴	103.93					101.96	101.06
2‴	71.99					71.17	70.73
3‴	73.40					73.21	73.10
4‴	71.52					68.19	68.36
5‴	76.00					72.39	71.05
6‴	61.70					61.10	60.94
$C_{acetalic}$		101.7	101.23				
		30.41					
CH_3–*C*H–Ph				78.70	78.82	78.73	
CH_3–CH–Ph				23.20	23.24	23.22	

TABLE II

^{1}H-N.M.R. DATA FOR β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1↔1)-α-D-Glc*p* (**1**)

Atom	*Chemical shift (δ scale)*			
	Unit A	*Unit B*	*Unit C*	*Unit D*
H-1	4.995 (7.92)[a]	4.909 (8.22)[a]	5.523 (3.69)[a]	5.508 (3.62)[a]
H-2	3.946	3.868	4.023	3.962
H-3	4.011	4.103	4.296	4.175
H-4	4.284	3.879	4.009	3.780
H-5	4.048	3.857	4.270	4.166
H-6	4.086	4.246	4.179	4.081
H-6′	4.141	4.274	4.234	4.184

[a] $^3J_{1,2}$ in Hz.

the ^{13}C-n.m.r. spectrum of which at 100 MHz contained 23 well separated signals, one (73.40 p.p.m.) with double intensity. The assignment of the most characteristic lines of the spectrum was straightforward, but the assignments of some lines were questionable on the basis of comparison with model compounds; hence, 2D-n.m.r. methods were used. After having assigned the ^{1}H-n.m.r. (400 MHz) spectrum of **1** with the aid of homonuclear shift-correlation (COSY) spectroscopy[20,21], various types of 2D heteronuclear shift-correlation experiments[22,23] were used to establish the assignments. The ^{13}C-n.m.r. data are given in Table I and the ^{1}H-n.m.r. data in Table II.

EXPERIMENTAL

General methods. — Melting points (uncorrected) were determined on a Kofler apparatus. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. ^{1}H- and ^{13}C-n.m.r. spectra were recorded with a Bruker WP-200 SY (^{1}H, 200 MHz) or 400 AM (^{1}H, 400 MHz) spectrometer for solutions in $CDCl_3$ (internal Me_4Si), or in D_2O (internal 1,4-dioxane, 67.4 p.p.m.). Reactions were monitored by t.l.c. on Kieselgel 60 F_{254} (Merck) with detection by charring with sulfuric acid. Kieselgel G (Reanal) was used for short-column chromatography.

2,3,2′,3′,4′,6′-Hexa-O-benzyl-4,6-O-(S)-(1-phenylethylidene)-α,α-trehalose (**2**). — A mixture of 2,3,2′,3′,4′,6′-hexa-*O*-benzyl-α,α-trehalose[10] (4.415 g, 5 mmol), acetophenone dimethyl acetal (14 mL), and toluene-*p*-sulphonic acid (100 mg) was stirred at room temperature. After 5 min, the mixture was diluted with CH_2Cl_2 (200 mL), washed with aqueous 5% $NaHCO_3$ (2 × 20 mL) and water (2 × 20 mL), dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (light petroleum–ethyl acetate, 8:2), to give **2** (4.36 g; 88.5%), isolated as a syrup, $[\alpha]_D$ +146° (*c* 0.4, chloroform).

Anal. Calc. for $C_{62}H_{64}O_{11}$: C, 75.59; H, 6.55. Found: C, 75.50; H, 6.59.

2,3,2′,3′,4′,6′-Hexa-O-benzyl-4,6-O-benzylidene-α,α-trehalose (**3**). — 2,3,2′,-

3',4',6'-Hexa-*O*-benzyl-α,α-trehalose[10] (400 mg) was treated with α,α-dimethoxytoluene, as described for the preparation of **2**. Column chromatography of the residue, using light petroleum–ethyl acetate (3:1), gave **3** (350 mg, 79.6%), isolated as a syrup, $[\alpha]_D$ +64° (*c* 1, chloroform).

Anal. Calc. for $C_{61}H_{62}O_{11}$: C, 75.44; H, 6.43. Found: C, 75.49; H, 6.39.

2,3,2',3',4',6'-Hexa-O-benzyl-6-O-(1-phenylethyl)-α,α-trehalose (**4**). — A solution of **2** (2.955 g; 3 mmol) in ether–dichloromethane (80 mL, 1:1) was treated with $LiAlH_4$ (1.20 g) and $AlCl_3$ (3.60 g) for 2 h at reflux temperature. After cooling, the excess of reagent was decomposed with ethyl acetate (3 mL) and $Al(OH)_3$ was precipitated with water. The mixture was diluted with ether (100 mL), filtered, washed with water (3 × 25 mL), dried, and concentrated. Column chromatography of the residue, using light petroleum–ethyl acetate (7:3), gave **4** (2.54 g, 86.8%), isolated as a syrup, $[\alpha]_D$ +96° (*c* 1.4, chloroform).

Anal. Calc. for $C_{62}H_{66}O_{11}$: C, 75.43; H, 6.74. Found: C, 75.37; H, 6.70.

2,3,4,2',3',4',6'-Hepta-O-benzyl-α,α-trehalose (**5**). — Compound **3** (270 mg) was hydrogenolysed with the $AlCl_3$ and $LiAlH_4$ reagent, as described for the preparation of **4**. Purification of the product by column chromatography then gave **5** (240 mg, 88.7%), $[\alpha]_D$ +95° (*c* 1, chloroform); lit.[10] $[\alpha]_D$ +96° (chloroform).

Anal. Calc. for $C_{61}H_{64}O_{11}$: C, 75.29; H, 6.63. Found: C, 75.21; H, 6.67.

O-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-(1→4)-2,3-di-O-benzyl-6-O-(1-phenylethyl)-α-D-glucopyranosyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranoside (**6**). — A mixture of **4** (493.6 mg; 0.5 mmol), $HgBr_2$ (180.2 mg; 0.5 mmol), dry dichloromethane (12 mL), and molecular sieves (4 Å, 1.5 g) was stirred for 1 h. 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (411.2 mg, 1 mmol) was added, and stirring was continued overnight at room temperature. The mixture was diluted with dichloromethane (150 mL), washed with aqueous 5% KI (2 × 25 mL), saturated aqueous $NaHCO_3$ (25 mL), and water (2 × 25 mL), dried (Na_2SO_4), and concentrated. Column chromatography of the residue yielded **6** (428 mg; 65%), isolated as a syrup, $[\alpha]_D$ +58° (*c* 1, chloroform).

Anal. Calc. for $C_{76}H_{84}O_{20}$: C, 69.29; H, 6.43. Found: C, 69.36; H, 6.40.

O-(3-O-Allyl-β-D-glucopyranosyl)-(1→4)-2,3-di-O-benzyl-6-O-(1-phenylethyl)-α-D-glucopyranosyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranoside (**7**). — Compound **4** (1.974 g, 2 mmol) was treated with 2,4,6-tri-*O*-acetyl-3-*O*-allyl-α-D-glucopyranosyl bromide[15] (1.228 g, 3 mmol) in the presence of $HgBr_2$ (720.8 mg, 2 mmol) for 30 h, as described for the preparation of **6**. Zemplén deacetylation and then column chromatography of the crude product gave **7** (1.57 g, 66%), $[\alpha]_D$ +87° (*c* 0.8, chloroform).

Anal. Calc. for $C_{71}H_{80}O_{16}$: C, 71.70; H, 6.78. Found: C, 71.79; H, 6.76.

O-(3-O-Allyl-2,4,6-tri-O-benzyl-β-D-glucopyranosyl)-(1→4)-2,3-di-O-benzyl-6-O-(1-phenylethyl)-α-D-glucopyranosyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranoside (**8**). — A mixture of **7** (1.10 g), powdered KOH (2.2 g), and benzyl chloride (15 mL) was stirred for 2 h at 100°, then cooled, diluted with CH_2Cl_2 (100 mL), filtered, and steam-distilled. After cooling, the residue was extracted with CH_2Cl_2 (3 × 40

mL), and the combined extracts were washed with water (2 × 25 mL), dried (Na_2SO_4), and concentrated. The product was purified by column chromatography (8:2 light petroleum–ethyl acetate) to give **8** (1.11 g, 82.2%), $[\alpha]_D$ +71° (*c* 0.9, chloroform).

Anal. Calc. for $C_{92}H_{98}O_{16}$: C, 75.70; H, 6.77. Found: C, 75.64; H, 6.80.

O-(2,4,6-Tri-O-benzyl-β-D-glucopyranosyl)-(1→4)-2,3-di-O-benzyl-6-O-(1-phenylethyl)-α-D-glucopyranosyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranoside (**9**). — A solution of **8** (980 mg) in 7:3:1 ethanol–toluene–water (22 mL) containing tris(triphenylphosphine)rhodium(I) chloride (250 mg) was boiled under reflux for 3 h, then cooled, and concentrated. A solution of the residue in 9:1 acetone–M hydrochloric acid (10 mL) was boiled for 10 min, then cooled, neutralised with aqueous $NaHCO_3$, and concentrated to dryness. Column chromatography (7:3 light petroleum–ethyl acetate) of the residue yielded syrupy **9** (670 mg, 70.3%).

Anal. Calc. for $C_{89}H_{94}O_{16}$: C, 75.30; H, 6.67. Found: C, 75.23; H, 6.71.

O-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-benzyl-β-D-glucopyranosyl)-(1→4)-2,3-di-O-benzyl-6-O-(1-phenylethyl)-α-D-glucopyranosyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranoside (**10**). — Methyl triflate (890 μL) was added to a stirred mixture of **9** (590 mg, 0.416 mmol), methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (471.8 mg, 1.247 mmol), dichloromethane (15 mL), and powdered molecular sieves (4 Å, 7.5 g) at room temperature. Triethylamine (2 mL) was added after 12 h, and the mixture was stirred for 10 min, filtered through a layer of Celite, and concentrated. Column chromatography (6:4 light petroleum–ethyl acetate) of the residue yielded **10** (523 mg, 71.9%), $[\alpha]_D$ +43° (*c* 0.9, chloroform).

Anal. Calc. for $C_{103}H_{112}O_{25}$: C, 70.69; H, 6.45. Found: C, 70.72; H, 6.42.

O-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-α-D-glucopyranosyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (**11**). — A mixture of **10** (360 mg), ethanol (30 mL), acetic acid (10 mL), and Pd/C (100 mg) was stirred under H_2 (1 atm.) for 2 days and then filtered. Insoluble material was washed with ethanol (2 × 10 mL), the combined filtrate and washings were concentrated, and the residue was acetylated conventionally with acetic anhydride (4 mL) in pyridine (10 mL) to yield **11** (249 mg, 96.4%). Crystallisation from 5 mL of ethanol gave material (152 mg, 58.9%) having m.p. 122–123°, $[\alpha]_D$ +65° (*c* 1, chloroform).

Anal. Calc. for $C_{52}H_{70}O_{35}$: C, 49.76; H, 5.62. Found: C, 49.79; H, 5.65.

O-β-D-Glucopyranosyl-(1→3)-O-β-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl α-D-glucopyranoside (**1**). — To a solution of **11** (110 mg) in dry methanol (20 mL) was added sodium methoxide (5 mg). The mixture was kept for 24 h at room temperature, then neutralised with Amberlite IR-120 (H^+) resin, filtered, and concentrated to give **1** (55 mg, 94.1%), $[\alpha]_D$ +97° (*c* 0.95, water).

Anal. Calc. for $C_{24}H_{42}O_{21}$: C, 43.24; H, 6.35. Found: C, 43.30; H, 6.30.

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