Carbohydrate Research, 164 (1987) 313–325
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

GLYCOSYLATED TREHALOSE. SYNTHESIS OF THE OLIGOSACCHARIDES OF THE GLYCOLIPID-TYPE ANTIGENS FROM *Mycobacterium smegmatis**

ZOLTÁN SZURMAI, JÁNOS KERÉKGYÁRTÓ, JÁNOS HARANGI, AND ANDRÁS LIPTÁK
Institute of Biochemistry, L. Kossuth University, P.O. Box 55, H-4010 Debrecen (Hungary)

(Received September 20th, 1986; accepted for publication, February 6th, 1987)

ABSTRACT

The oligosaccharide components, 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* (**1**) and β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→6)-α-D-Glc*p*-(1↔1)-α-D-Glc*p*, of the glycolipid-type antigens isolated from *M. smegmatis* have been synthesised from 2,3,4,2′,3′,4′,6′-hepta-*O*-acetyl-α,α-trehalose and the appropriate glycosyl bromides under Helferich conditions with $Hg(CN)_2$ as the promoter. Condensation of the trisaccharide glycosyl bromide **27** gave an orthoester derivative (**28**) which could be rearranged, using $HgBr_2$ or boron trifluoride etherate, into the acetylated derivative (**29**) of **1**. The model compound β-D-Glc*p*-(1→6)-α-D-Glc*p*-(1↔1)-α-D-Glc*p* has also been synthesised.

INTRODUCTION

The surface antigens of mycobacteria are glycolipids[1] or peptidoglycolipids[2]. The carbohydrate moieties of these antigens are unusual in that they contain rare sugars, such as 6-deoxy-L-talose, and their common monosaccharide units carry *O*-methyl and/or *O*-acyl groups. Recently, the glycosylated α,α-trehaloses **1** and **2**, the oligosaccharide moieties of glycolipid-type antigens, have been isolated[3] from *M. smegmatis*. Other glycosylated trehaloses have been reported, namely, 4-*O*-β-laminaribiosyl-α,α-trehalose from *M. kansasii*[4], 3-*O*-α-D-glucopyranosyl-α,β-trehalose from *Streptococcus faecalis*[5], and 2-*O*-α-D-glucopyranosyl-α,α-trehalose from *Selaginella kraussinana* (an unpublished finding of Fischer and Kandler cited by Elbein[6]). The presence of penta-[3] and tetra-saccharides[3,4] in the antigens of various human pathogenic mycobacteria has attracted attention and emphasises the biological importance of these types of oligosaccharides.

Trehalose is an abundant natural disaccharide in the plant kingdom and in bacteria and micro-organisms[6,7]. Of the many syntheses published[8–14], only two[13,14] gave good yields and these were only moderately stereoselective. The synthesis of

*Dedicated to Burckhardt Helferich in commemoration of the centenary of his birth.

0008-6215/87/$ 03.50

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*

1

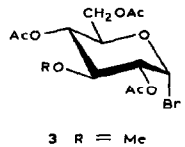
β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→6)-α-D-Glc*p*-(1↔1)-α-D-Glc*p*

2

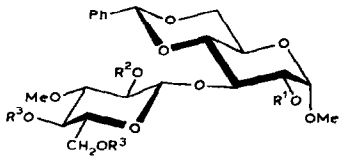
non-reducing oligosaccharides requires a suitable glycosyl donor that provides the desired anomeric configuration and control of the anomeric configuration of the glycosyl acceptor in which HO-1 is unsubstituted. The first requirement can usually be met, but there appears not to be a suitable method[15] to meet the second requirement. Hence, 2,3,4,2′,3′,4′,6′-hepta-*O*-acetyl-α,α-trehalose was used for the syntheses of **1** and **2**.

RESULTS AND DISCUSSION

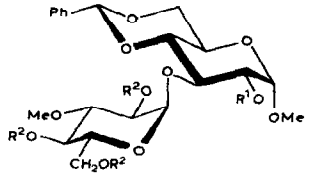
The 3 + 2 block synthesis of **1** required a suitable glycosyl bromide derivative of 3-*O*-Me-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→4)-D-Glc*p*, which was synthesised as follows. Condensation of 2,4,6-tri-*O*-acetyl-3-*O*-methyl-α-D-glucopyranosyl bromide[16] (**3**) with methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside[17] under Helferich[18] conditions gave a mixture of **4** and **5**. The β anomer **4** was the major product (61%) and 4.4% of the α anomer **5** was also isolated. Under



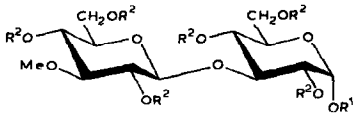
3 R = Me
14 R = Allyl



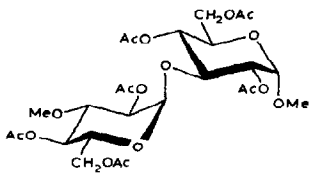
4 R^1 = Bz, R^2 = R^3 = Ac
6 R^1 = Bz, R^2 = Ac, R^3 = H
7 R^1 = R^2 = R^3 = H



5 R^1 = Bz, R^2 = Ac
8 R^1 = R^2 = H



9 R^1 = Me, R^2 = Ac
11 R^1 = R^2 = Ac
12 R^1 = Me, R^2 = H
13 R^1 = R^2 = H

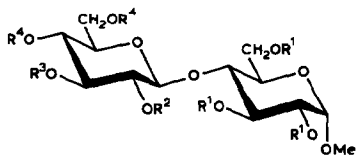


10

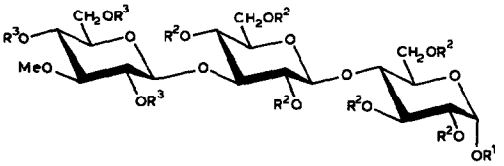
Zemplén conditions, BzO-2 and AcO-2′ of **4** had not been removed after 4 h and amorphous **6** could be isolated. Similar sterically hindered deacylations have been reported[19–21]. However, after 2 days, saponification of **4** and **5** was complete and crystalline **7** and **8**, respectively, were isolated. A one-pot reaction was used for the reaction sequence **3**→**4** + **5**→**7** + **8**→**9** + **10**, and the minor product **10** was also isolated. Prolonged treatment of **9** with methanolic sodium methoxide afforded methyl 3′-*O*-methyl-α-laminaribioside (**12**). Acetolysis of **9** gave 1,2,4,6,2′,4′,6′-hepta-*O*-acetyl-3′-*O*-methyl-α-laminaribiose (**11**), saponification of which gave 3′-*O*-methyl-laminaribiose (**13**). Attempts to glycosylate methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside, using either **11** in the presence of trimethylsilyl triflate or 3′-*O*-methyl-α-acetobromolaminaribiose in the presence of various promoters, failed. The low reactivity of this glycosyl acceptor is known[22,23] but, in the light of our experiments, it is assumed that acetobromolaminaribiose or its 3′-*O*-methyl derivative are rather sluggish glycosyl donors. Hence, an alternative approach to **1** was investigated.

Glycosylation of methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside[24] with 2,4,6-tri-*O*-acetyl-3-*O*-allyl-α-D-glucopyranosyl bromide[21] (**14**) at room temperature under Helferich conditions was extremely slow, but proceeded smoothly at 60° to give 73% of **15**. Under Zemplén deacetylation conditions for 1 h at room temperature, **15** gave the 2′-acetate **16** but, at the boiling point, the fully deacetylated product **17** was obtained. The hexa-*O*-benzyl derivative (**18**) of **17** was *O*-deallylated using tris(triphenylphosphine)rhodium(I) chloride[25,26] as catalyst, to yield 78% of **19**. Glycosylation of **19** with **3** gave 90% of the trisaccharide derivative **20**. Hydrogenolysis (Pd/C) of **20** and acetylation of the product afforded the acetylated methyl glycoside (**21**) of the target trisaccharide. Acetolysis of **21** gave the α-trisaccharide deca-acetate **22**, and the glycosyl bromide (**27**) of **22** was used to synthesise the desired pentasaccharide.

In order to convert α,α-trehalose into a suitable glycosyl acceptor, it was treated[27] with 1.2 equiv. of trityl chloride followed by acetylation to give **23** (24% after fractional crystallisation). Detritylation of **23** with aqueous 80% acetic acid afforded **24**, having HO-6 unsubstituted.



15 R^1 = Bzl, R^2 = R^4 = Ac, R^3 = Allyl
16 R^1 = Bzl, R^2 = Ac, R^3 = Allyl, R^4 = H
17 R^1 = Bzl, R^2 = R^4 = H, R^3 = Allyl
18 R^1 = R^2 = R^4 = Bzl, R^3 = Allyl
19 R^1 = R^2 = R^4 = Bzl, R^3 = H

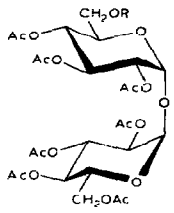


20 R^1 = Me, R^2 = Bzl, R^3 = Ac
21 R^1 = Me, R^2 = R^3 = Ac
22 R^1 = R^2 = R^3 = Ac
27 R^1 = Br, R^2 = R^3 = Ac

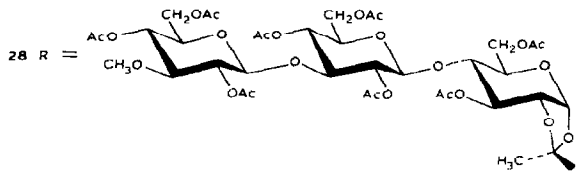
The reactivity of **24** was established by using acetobromoglucose as the glycosyl donor and $Hg(CN)_2$ as the catalyst, which gave 95% of the β product **25**; no trace of the α anomer could be detected. The ^{13}C-n.m.r. spectrum of **25** accorded with the structure assigned. Thus, C-1 of the newly formed β-D-glucopyranosyl unit resonated at 100.52 p.p.m., the other two anomeric carbons at 92.20 and 91.92 p.p.m.. and the glucosylated C-6 of trehalose at 67.47 p.p.m. A similar result was obtained from the reaction of **24** with α-acetobromocellobiose, and 80% of the acetylated tetrasaccharide **26** was obtained. The ^{13}C-n.m.r. spectrum of **26** contained signals for anomeric carbons of the β-D-glucopyranosyl units at 100.56 and 100.36 p.p.m. and for those of the trehalose moiety at 92.13 and 91.92 p.p.m. The glycosylated C-6 of the trehalose unit resonated at 67.43 p.p.m.

The coupling of **24** and **27** proceeded smoothly to give 61.7% of a product whose ^{13}C-n.m.r. spectrum contained signals for a quaternary carbon (121.07 p.p.m.) and five anomeric carbons (101.66, 100.94, 96.78, 92.23, and 92.13 p.p.m.). The resonance of the substituted C-6 of the trehalose unit appeared at high field (63.20 p.p.m.) and there was a non-acetyl methyl signal at 19.89 p.p.m. These data are consistent with the orthoester structure **28**. The ^1H-n.m.r. spectrum of **28** contained a 3-proton singlet at 1.65 p.p.m. (*endo*-Me of the orthoester) and a 1-proton doublet at 5.66 p.p.m. ($J_{1,2}$ 3.2 Hz) assigned to the anomeric proton of the orthoester. The orthoester structure **28** was also confirmed by a simple acid hydrolysis test[28].

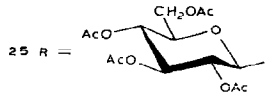
Treatment of the orthoester **28** with $HgBr_2$ (ref. 29) or with boron trifluoride etherate[30] gave the desired acetylated pentasaccharide **29**, the ^{13}C-n.m.r. spectrum



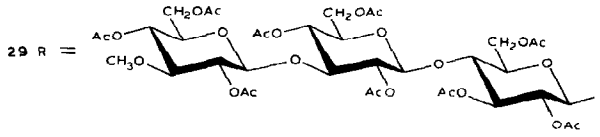
23 R = Tr
24 R = H



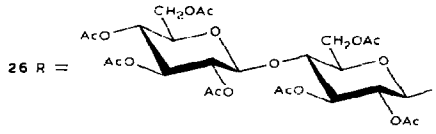
28 R =



25 R =



29 R =



26 R =

β-D-Glcp-(1→6)-α-D-Glcp-(1↔1)-α-D-Glcp

30

TABLE I

^{13}C-N.M.R. DATA[a]

		1	2	30	12	13	
						α	β
C-1	A[b]	93.55	93.70	93.70			
C-2		71.18	71.30	71.31			
C-3		72.99	72.91	72.90			
C-4		69.91	69.82	69.98			
C-5		71.87	71.54	71.53			
C-6		60.79	60.94	60.92			
C-1	B	93.55	93.51	93.70			
C-2		71.18	71.30	71.31			
C-3		72.76	72.82	72.83			
C-4		69.91	69.82	69.98			
C-5		72.40	72.54	72.51			
C-6		68.47	68.62	68.59			
C-1	C	102.48[b]	102.63[b]	102.96			
C-2		73.13	73.21	73.43			
C-3		74.90	75.63	76.03			
C-4		78.89	79.08	69.81			
C-5		75.78	75.89	76.18			
C-6		60.79	60.72	61.10			
C-1	D	102.65[b]	102.78[b]		99.33	92.28	95.98
C-2		73.13	73.50		70.91	69.21	74.04
C-3		84.29	76.04		82.85	82.78	85.09
C-4		69.62	69.82		68.28	68.47	69.38
C-5		76.03	76.28		71.59	71.53	75.84
C-6		60.79	60.94		60.78	60.92	60.92
C-1	E	102.88[b]			103.01	103.04	
C-2		73.13			73.03	73.11	
C-3		85.27			85.14	85.38	
C-4		69.12			69.15	69.21	
C-5		76.03			76.02	76.12	
C-6		60.79			60.78	60.92	
CH_3O-1		—			55.21	—	
CH_3O-3		59.88			59.96	59.93	

E D C B

[a]At 50 MHz, in D_2O and 27°. [b]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*. [c]Assignments may be interchanged.
A

of which accorded with the proposed structure.

Deacetylation of **25** and **26** gave the oligosaccharides **30** and **2**, respectively. Removal of the acetyl groups from **29** required longer treatment (~48 h) in order to obtain the pentasaccharide **1**.

The ^{13}C-n.m.r. spectra (Table I) of **1**, **2**, and **30** verified their structure, and the assignments were based on comparisons with data for suitable model compounds[31].

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 spectrometer for solutions in $CDCl_3$ (internal Me_4Si), D_2O (internal 1,4-dioxane), or $(CD_3)_2SO$. Reactions were monitored by t.l.c. on Kieselgel 60 F_{254} (Merck) with detection by charring with sulfuric acid. Both Kieselgel G and Kieselgel H (Reanal) were used for short-column chromatography.

Methyl 2-O-benzoyl-4,6-O-benzylidene-3-O-(2,4,6-tri-O-acetyl-3-O-methyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**4**) *and methyl 2-O-benzoyl-4,6-O-benzylidene-3-O-(2,4,6-tri-O-acetyl-3-O-methyl-α-D-glucopyranosyl)-α-D-glucopyranoside* (**5**). — To a solution of methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (3.86 g, 10 mmol) in benzene–nitromethane (1:1, 100 mL) was added $Hg(CN)_2$ (3.03 g, 12 mmol), and 50 mL of the solvent was distilled off at atmospheric pressure. The mixture was cooled to 60°, 2,4,6-tri-*O*-acetyl-3-*O*-methyl-α-D-glucopyranosyl bromide[16] (**3**; 4.598 g, 12 mmol) was added, and the mixture was stirred at 60°. After 3 h, more $Hg(CN)_2$ (0.758 g, 3 mmol) and **3** (1.15 g, 3 mmol) were added and stirring was continued for 2 h. The mixture was then concentrated, diluted with dichloromethane (350 mL), washed with aqueous 5% KI (2 × 35 mL) and water (2 × 35 mL), dried (Na_2SO_4), and concentrated. Column chromatography of the residue gave amorphous **4** (4.21 g, 61.1%), $[\alpha]_D$ +24° (*c* 0.7, chloroform), R_F 0.22 (9:1 CH_2Cl_2–EtOAc). ^{1}H-N.m.r. data ($CDCl_3$): δ 8.15–7.25 (m, 10 H, 2 Ph), 5.60 (s, 1 H, PhC*H*), 5.15–4.92 (m, 3 H, H-2,2′,4′), 5.03 (d, 1 H, H-1), 4.70 (d, 1 H, H-1′), 3.38 (s, 3 H, OMe), 3.21 (s, 3 H, OMe), 2.06, 1.97, 1.65 (3 s, each 3 H, 3 OAc); $J_{1',2'}$ 8.0, $J_{1,2}$ 3.5 Hz.

Anal. Calc. for $C_{34}H_{40}O_{15}$: C, 59.30; H, 5.85. Found: C, 59.48; H, 5.90.

Further elution gave amorphous **5** (300 mg, 4.36%), $[\alpha]_D$ +89° (*c* 0.7, chloroform), R_F 0.27 (9:1 CH_2Cl_2–EtOAc). ^{1}H-N.m.r. data ($CDCl_3$): δ 8.15–7.30 (m, 10 H, 2 Ph), 5.54 (s, 1 H, PhC*H*), 5.51 (d, 1 H, H-1′), 4.96 (d, 1 H, H-1), 3.41 (s, 3 H, OMe), 3.34 (s, 3 H, OMe), 2.03, 1.86, 1.75 (3 s, each 3 H, 3 OAc); $J_{1,2}$ 3.8, $J_{1',2'}$ 3.5 Hz.

Anal. Found: C, 59.60; H, 5.78.

A mixture (870 mg, 12.6%) of **4** and **5** was also isolated.

Methyl 3-O-(2-O-acetyl-3-O-methyl-β-D-glucopyranosyl)-2-O-benzoyl-4,6-O-benzylidene-α-D-glucopyranoside (**6**). — To a solution of **4** (0.4 g) in dry methanol (20 mL) was added sodium methoxide (5 mg). The mixture was stored for 4 h at room temperature, then neutralised with Amberlite IR-120 (H^+) resin, filtered, and concentrated. Column chromatography of the residue (315 mg) gave

amorphous **6** (173 mg, 49.3%), $[\alpha]_D$ +64° (*c* 0.9, chloroform), R_F 0.38 (95:5 CH_2Cl_2–MeOH). ^{1}H-N.m.r. data ($CDCl_3$): δ 8.20–7.35 (m, 10 H, 2 Ph), 5.55 (s, 1 H, PhC*H*), 5.10 (dd, 1 H, H-2), 5.02 (d, 1 H, H-1), 4.86 (dd, 1 H, H-2'), 4.68 (d, 1 H, H-1'), 3.40 (s, 3 H, OMe), 3.33 (s, 3 H, OMe), 1.67 (s, 3 H, OAc); $J_{1,2}$ 3.5, $J_{1',2'}$ 7 Hz.

Anal. Calc. for $C_{30}H_{36}O_{13}$: C, 59.60; H, 6.00. Found: C, 60.10; H, 6.08.

*Methyl 4,6-*O*-benzylidene-3-*O*-(3-*O*-methyl-β-*D*-glucopyranosyl)-α-*D*-glucopyranoside* (**7**). — Saponification of **4** (2.9 g), as described above but for 2 days, afforded **7** (1.62 g, 83.9%). Crystallisation from ethanol (13 mL) gave material (645 mg, 33.4%) having m.p. 196–199°, $[\alpha]_D$ +33° (*c* 1, methanol), R_F 0.38 (9:1 CH_2Cl_2–MeOH). ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 7.55–7.30 (m, 5 H, Ph), 5.59 (s, 1 H, PhC*H*), 5.34, 5.20, 5.02 (3 d, each 1 H, 3 OH), 4.69 (d, 1 H, H-1), 4.52 (d, 1 H, H-1'), 3.44 (s, 3 H, OMe), 3.34 (s, 3 H, OMe); $J_{1,2}$ 3.8, $J_{1',2'}$ 7.6 Hz.

Anal. Calc. for $C_{21}H_{30}O_{11}$: C, 55.02; H, 6.60. Found: C, 55.12; H, 6.56.

*Methyl 4,6-*O*-benzylidene-3-*O*-(3-*O*-methyl-α-*D*-glucopyranosyl)-α-*D*-glucopyranoside* (**8**). — Saponifiation of **5** (160 mg), as described above but for 2 days, and column chromatography of the product gave **8** (75 mg, 70.4%), m.p. 216–220° (from ethanol), $[\alpha]_D$ +126.5° (*c* 0.2, methanol), R_F 0.31 (9:1 CH_2Cl_2–MeOH). ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 7.55–7.31 (m, 5 H, Ph), 5.62 (s, 1 H, PhC*H*), 5.10 (bs, 1 H, H-1'), 4.69 (d, 1 H, H-1), 3.50 (s, 3 H, OMe), 3.34 (s, 3 H, OMe); $J_{1,2}$ 3.6 Hz.

Anal. Calc. for $C_{21}H_{30}O_{11}$: C, 55.02; H, 6.60. Found: C, 54.92; H, 6.59.

*Methyl 2,4,6-tri-*O*-acetyl-3-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-methyl-β-*D*-glucopyranosyl)-α-*D*-glucopyranoside* (**9**) *and methyl 2,4,6-tri-*O*-acetyl-3-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-methyl-α-*D*-glucopyranosyl)-α-*D*-glucopyranoside* (**10**). — Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-*D*-glucopyranoside (3.86 g, 10 mmol) was reacted with **3**, and the product was deacetylated as described above for the preparation of **4** and **5**. A solution of the product in CH_2Cl_2 (200 mL) was washed with water (20 mL), dried, and concentrated, and the residue was hydrolysed with boiling aqueous 80% acetic acid for 10 min. The mixture was concentrated and toluene (3 × 15 mL) was evaporated from the residue, which was then treated conventionally with acetic anhydride (30 mL) and pyridine (30 mL). Column chromatography (Kieselgel G) of the product yielded **10** (375 mg, 6%), m.p. 117–119° (from ethanol), $[\alpha]_D$ +131° (*c* 0.8, chloroform), R_F 0.24 (8:2 CH_2Cl_2–EtOAc). ^{1}H-N.m.r. data ($CDCl_3$): δ 5.25 (d, 1 H, H-1'), 5.22–4.65 (m, 5 H, H-1,2,4,2',4'), 4.32–3.53 (m, 8 H, skeleton protons), 3.44 (s, 3 H, OMe), 3.37 (s, 3 H, OMe), 2.15–2.01 (m, 18 H, 6 OAc); $J_{1',2'}$ 3.8 Hz.

Anal. Calc. for $C_{26}H_{38}O_{17}$: C, 50.16; H, 6.15. Found: C, 50.20; H, 6.18.

Eluted second was a mixture (975 mg, 15.7%) of **9** + **10**. Eluted third was **9** (2.75 g, 44.2%), m.p. 154° (from ethanol), $[\alpha]_D$ +41° (*c* 1, chloroform), R_F 0.20 (8:2 CH_2Cl_2–EtOAc).

Anal. Found: C, 50.28; H, 6.12.

*1,2,4,6-Tetra-*O*-acetyl-3-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-methyl-β-*D*-glucopyranosyl)-α,β-*D*-glucopyranose* (**11**). — To a solution of **9** (1.0 g) in acetic anhydride (2

mL) was slowly added 4% (v/v) sulfuric acid in acetic anhydride (2 mL) at 0°. The mixture was stirred for 4 h at room temperature, then poured into ice–water containing $NaHCO_3$, and extracted with CH_2Cl_2 (3 × 30 mL). The combined extracts were washed with saturated aqueous $NaHCO_3$ (15 mL) and water (3 × 20 mL), then dried, and concentrated. Column chromatography (Kieselgel G) of the residue yielded **11** (950 mg, 90.9%), isolated as a foam, $[\alpha]_D$ +19° (*c* 0.8, chloroform), R_F 0.20 (8:2 CH_2Cl_2–EtOAc). ^{1}H-N.m.r. data ($CDCl_3$): δ 6.25 (d, ~0.85 H, H-1α), 5.63 (d, ~0.15 H, H-1β), 4.57 (d, 1 H, H-1′), 3.37 (s, 3 H, OMe), 2.22–2.00 (m, 21 H, 7 OAc); $J_{1,2\alpha}$ 3.5, $J_{1,2\beta}$ 7, $J_{1',2'}$ ~7 Hz.

Anal. Calc. for $C_{27}H_{38}O_{18}$: C, 49.85; H, 5.89. Found: C, 49.68; H, 5.93.

Methyl 3-O-(3-O-methyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**12**). — To a solution of **9** (600 mg) in dry methanol (60 mL) was added sodium methoxide (20 mg). The solution was stored for 2 days at room temperature, then neutralised with Amberlite IR-120 (H^+) resin, filtered, and concentrated. Column chromatography (Kieselgel H) of the residue (320 mg, 89.7%), using 2:1:1 1-butanol–methanol–water, gave amorphous **12** (275 mg, 77%); $[\alpha]_D$ +69° (*c* 1.1, water).

Anal. Calc. for $C_{14}H_{26}O_{11}$: C, 45.40; H, 7.08. Found: C, 45.60; H, 7.15.

O-(3-O-Methyl-β-D-glucopyranosyl)-(1→3)-D-glucopyranose (**13**). — Saponification of **11** (0.2 g), as described for **12**, and column chromatography (Kieselgel H; 2:1:1 1-butanol–methanol–water) of the product (93 mg, 84.9%) yielded amorphous **13** (62 mg, 56.6%), $[\alpha]_D$ +19° (equil., *c* 0.5, water).

Anal. Calc. for $C_{13}H_{24}O_{11}$: C, 43.82; H, 6.79. Found: C, 44.01; H, 6.84.

Methyl 2,3,6-tri-O-benzyl-4-O-(2,4,6-tri-O-acetyl-3-O-allyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**15**). — Methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside[24] (2.6 g) was treated with 2,4,6-tri-*O*-acetyl-3-*O*-allyl-α-D-glucopyranosyl bromide[21] (**14**) as described for the preparation of **4** and **5**. Column chromatography of the product (97:3 CH_2Cl_2–acetone) afforded syrupy **15** (3.24 g, 73%), $[\alpha]_D$ −3° (*c* 0.6, chloroform), R_F 0.22. ^{1}H-N.m.r. data ($CDCl_3$): δ 7.45–7.20 (m, 15 H, 3 Ph), 5.97–5.64 (m, 1 H, –CH=), 3.38 (s, 3 H, OMe), 2.06, 2.01, 1.97 (3 s, each 3 H, 3 OAc).

Anal. Calc. for $C_{43}H_{52}O_{14}$: C, 65.14; H, 6.61. Found: C, 65.32; H, 6.56.

Methyl 4-O-(2-O-acetyl-3-O-allyl-β-D-glucopyranosyl)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**16**). — Compound **15** (396.4 mg, 0.5 mmol) was saponified at room temperature for 1 h as described for the synthesis of **6**. Column chromatography (8:2 CH_2Cl_2–acetone) of the product yielded amorphous **16** (278 mg, 78.4%), $[\alpha]_D$ +19° (*c* 0.3, chloroform), R_F 0.54. ^{1}H-N.m.r. data ($CDCl_3$): δ 7.45–7.20 (m, 15 H, 3 Ph), 5.97–5.75 (m, 1 H, –CH=), 3.38 (s, 3 H, OMe), 2.00 (s, 3 H, OAc).

Anal. Calc. for $C_{39}H_{48}O_{12}$: C, 66.09; H, 6.83. Found: C, 66.18; H, 6.75.

Methyl 4-O-(3-O-allyl-β-D-glucopyranosyl)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**17**). — To a solution of **15** (2.40 g) in dry methanol (160 mL) was added sodium methoxide (80 mg). The mixture was boiled under reflux for 6 h, then neutralised with Amberlite IR-120 (H^+) resin, and concentrated. The amorphous product (1.82 g, 90.2%), which was used for the next step without

further purification, had $[\alpha]_D$ +44° (*c* 0.1, chloroform), R_F 0.42 (8:2 CH_2Cl_2–acetone). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.45–7.20 (m, 15 H, 3 Ph), 6.08–5.85 (m, 1 H, –CH=), 3.38 (s, 3 H, OMe).

Methyl 4-O-(3-O-allyl-2,4,6-tri-O-benzyl-β-D-glucopyranosyl)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (**18**). — A mixture of **17** (1.60 g), powdered KOH (2 g), and benzyl chloride (20 mL) was stirred at 100° for 4 h, then cooled, diluted with CH_2Cl_2 (100 mL), filtered, and steam-distilled. After cooling, the residue was extracted with CH_2Cl_2 (3 × 50 mL), the combined extracts were washed with water (2 × 30 mL), dried, and concentrated. Column chromatography (Kieselgel G, 95:5 CH_2Cl_2–ethyl acetate) of the product gave **18** (1.71 g; 76%), which, after recrystallisation from cyclohexane, had m.p. 104–105°, $[\alpha]_D$ +26° (*c* 0.9, chloroform), R_F 0.49 (95:5 CH_2Cl_2–EtOAc). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.45–7.15 (m, 30 H, 6 Ph), 6.03–5.82 (m, 1 H, –CH=), 3.38 (s, 3 H, OMe).

Anal. Calc. for $C_{58}H_{64}O_{11}$: C, 74.34; H, 6.88. Found: C, 74.36; H, 6.91.

Methyl 2,3,6-tri-O-benzyl-4-O-(2,4,6-tri-O-benzyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**19**). — (*a*) A mixture of **18** (450 mg) and 10% Pd/C (200 mg) in 2:1:1 acetic acid–methanol–water (20 mL) was boiled under reflux for 3 h, then cooled, and filtered. The insoluble material was washed with methanol (2 × 20 mL), and the combined filtrate and washings were concentrated. Column chromatography (Kieselgel G, 95:5 CH_2Cl_2–ethyl acetate) of the residue yielded syrupy **19** (125 mg, 29.0%), $[\alpha]_D$ +26° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.45–7.15 (m, 30 H, 6 Ph), 3.37 (s, 3 H, OMe), 2.34 (bs, 1 H, OH).

Anal. Calc. for $C_{55}H_{60}O_{11}$: C, 73.64; H, 6.74. Found: C, 73.40; H, 6.62.

(*b*) A solution of **18** (1 g) in 7:3:1 ethanol–toluene–water (20 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 of the residue (95:5 CH_2Cl_2–ethyl acetate) gave syrupy **19** (745 mg, 77.8%).

Methyl 2,3,6-tri-O-benzyl-4-O-[2,4,6-tri-O-benzyl-3-O-(2,4,6-tri-O-acetyl-3-O-methyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]-α-D-glucopyranoside (**20**). — Compound **19** (0.8 g, 0.89 mmol) was treated with **3** at 65° as described for the preparation of **4** and **5**. Column chromatography (Kieselgel G, 95:5 CH_2Cl_2–acetone) of the product gave syrupy **20** (965 mg, 90.2%), $[\alpha]_D$ +9° (*c* 0.4, chloroform), R_F 0.41 (95:5 CH_2Cl_2–acetone). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.45–7.15 (m, 30 H, 6 Ph), 3.45 (s, 3 H, OMe), 3.37 (s, 3 H, OMe), 2.18 (s, 3 H, OAc), 2.11 (s, 6 H, 2 OAc).

Anal. Calc. for $C_{68}H_{78}O_{19}$: C, 68.10; H, 6.56. Found: C, 68.21; H, 6.49.

Methyl 2,3,6-tri-O-acetyl-4-O-[2,4,6-tri-O-acetyl-3-O-(2,4,6-tri-O-acetyl-3-O-methyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]-α-D-glucopyranoside (**21**). — A mixture of **20** (800 mg), ethanol (15 mL), acetic acid (5 mL) and 10% Pd/C (200 mg) was stirred under H_2 (1 atm.) for 4 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 (10 mL) in pyridine (10 mL), to give **21** (542 mg, 89.2%). Recrystallisation from ethanol gave material (280 mg, 46.1%) having m.p. 222–223°, $[\alpha]_D$ +16° (*c* 0.7, chloroform), R_F 0.31 (85:15 CH_2Cl_2–acetone). ^{1}H-N.m.r. data ($CDCl_3$): δ 3.42 (s, 3 H, OMe), 3.38 (s, 3 H, OMe), 2.25–1.95 (m, 27 H, 9 OAc).

Anal. Calc. for $C_{38}H_{54}O_{25}$: C, 50.10; H, 5.97. Found: C, 49.99; H, 5.96.

1,2,3,6-Tetra-O-acetyl-4-O-[2,4,6-tri-O-acetyl-3-O-(2,4,6-tri-O-acetyl-3-O-methyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]-α-D-glucopyranose (**22**). — Acetolysis of **21** (400 mg), as described for the preparation of **11**, yielded **22** (336 mg, 81.5%) which, after recrystallisation from ethanol, had m.p. 214–217°, $[\alpha]_D$ +4° (*c* 0.7, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 6.26 (d, 1 H, H-1), 3.36 (s, 3 H, OMe); $J_{1,2}$ 3.6 Hz.

Anal. Calc. for $C_{39}H_{54}O_{26}$: C, 49.89; H, 5.80. Found: C, 49.76; H, 5.73.

Tritylation of α,α-trehalose. — A mixture of α,α-trehalose dihydrate (2.0 g, 5.29 mmol) and dry ethanol (20 mL) was boiled under reflux for 30 min and then concentrated to dryness. To a solution of the residue in dry pyridine (20 mL) was added trityl chloride (1.916 g, 6.87 mmol). The mixture was stirred at 40° for 2 days and then cooled, and acetic anhydride (10 mL) was added. Conventional work-up and column chromatography (9:1 CH_2Cl_2–EtOAc) of the product gave, first, 2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-di-*O*-trityl-α,α-trehalose (2.70 g, 47.3%), m.p. 246–248° (from ethanol), $[\alpha]_D$ +112° (*c* 1, chloroform), R_F 0.61 (9:1 CH_2Cl_2–EtOAc). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.50–7.15 (m, 30 H, 6 Ph), 5.55–5.00 (m, 8 H, H-1,2,3,4,1′,2′,3′,4′), 4.20–3.98 (m, 2 H, H-5,5′), 3.09 (m, 4 H, H-6,6,6′,6′), 2.01, 1.91, 1.76 (3 s, each 6 H, 6 OAc).

Anal. Calc. for $C_{62}H_{62}O_{17}$: C, 69.01; H, 5.79. Found: C, 69.12; H, 5.73.

Eluted second was 2,3,4,2′,3′,4′,6′-hepta-*O*-acetyl-6-*O*-trityl-α,α-trehalose (**23**; 1.11 g, 23.9%), m.p. 203–205° (from ethanol), $[\alpha]_D$ +134° (*c* 0.7, chloroform), R_F 0.18 (9:1 CH_2Cl_2–EtOAc). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.45–7.15 (m, 15 H, 3 Ph), 5.55–5.00 (m, 8 H, H-1,2,3,4,1′,2′,3′,4′), 4.32–4.00 (m, 4 H, H-5,6,6,5′), 3.06 (m, 2 H, H-6′,6′), 2.15–1.75 (m, 21 H, 7 OAc).

Anal. Calc. for $C_{45}H_{50}O_{18}$: C, 61.50; H, 5.73. Found: C, 61.48; H, 5.70.

2,3,4,6,2′,3′,4′-Hepta-O-acetyl-α,α-trehalose (**24**). — A solution of **23** (1.0 g) in aqueous 80% acetic acid (25 mL) was stirred at 80° for 1 h, then cooled, diluted with water, and extracted with CH_2Cl_2. The combined extracts were washed with saturated aqueous $NaHCO_3$ and water, then dried (Na_2SO_4), and concentrated to give **24** (505 mg, 69.7%), m.p. 124–126° (from ethanol), $[\alpha]_D$ +167.5° (*c* 0.7, chloroform), R_F 0.50 (8:2 CH_2Cl_2–acetone). ^{1}H-N.m.r. data ($CDCl_3$): δ 5.62–4.94 (m, 8 H, skeleton protons), 4.32–3.85 (m, 4 H, H-5,6,6,5′), 3.62 (m, 2 H, H-6′,6′), 2.22 (t, 1 H, OH), 2.15–2.00 (m, 21 H, 7 OAc).

Anal. Calc. for $C_{26}H_{36}O_{18}$: C, 49.06; H, 5.70. Found: C, 49.10; H, 5.73.

2,3,4,2′,3′,4′,6′-Hepta-O-acetyl-6-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-α,α-trehalose (**25**). — To a solution of **24** (254.6 mg, 0.4 mmol) in benzene–nitromethane (1:1, 30 mL) was added $Hg(CN)_2$ (121.3 mg, 0.48 mmol), and 15 mL

of the solvent was distilled off at atmospheric pressure. The mixture was cooled to 40°, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (acetobromoglucose) (197 mg, 0.48 mmol) was added, and the mixture was stirred at 40°. After 3 h, more $Hg(CN)_2$ (50.5 mg, 0.2 mmol) and acetobromoglucose (82.2 mg, 0.2 mmol) were added, and stirring was continued for 3 h. The mixture was then concentrated, diluted with CH_2Cl_2 (80 mL), washed with aqueous 5% KI (2 × 10 mL), and water (3 × 10 mL), dried (Na_2SO_4), and concentrated. Column chromatography (8:2 CH_2Cl_2–acetone) of the residue gave **25** (370 mg, 95.6%), isolated as a foam, $[\alpha]_D$ +92° (*c* 0.5, chloroform), R_F 0.72 (8:2 CH_2Cl_2–acetone).

Anal. Calc. for $C_{40}H_{54}O_{27}$: C, 49.66; H, 5.62. Found: C, 49.71; H, 5.68.

*2,3,4,2',3',4',6'-Hepta-*O*-acetyl-6-*O*-(2,3,6,2',3',4',6'-hepta-*O*-acetyl-β-cellobiosyl)-α,α-trehalose* (**26**). — Compound **24** (0.2 g) was glycosylated with acetobromocellobiose at 60°, as described for **25**. Work-up and column chromatography (8:2 CH_2Cl_2–acetone) of the product yielded **26** (315 mg, 79.9%), isolated as a foam, $[\alpha]_D$ +72° (*c* 0.5, chloroform).

Anal. Calc. for $C_{52}H_{70}O_{35}$: C, 49.76; H, 5.62. Found: C, 49.80; H, 5.64.

*2,3,6-Tri-*O*-acetyl-4-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-methyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]-α-D-glucopyranosyl bromide* (**27**). — To a solution of **22** (0.27 g) in dry CH_2Cl_2 (4 mL) was added 33% HBr in glacial acetic acid (2 mL), and the mixture was kept at 5° for 1 h. Conventional work-up gave **27** (265 mg, 96%), $[\alpha]_D$ +46° (*c* 1, chloroform), which was used directly for the next step without further purification.

*Reaction of hepta-*O*-acetyl-α,α-trehalose* (**24**) *with bromide* **27**. — To a solution of **24** (130 mg, 0.204 mmol) in 1:1 benzene–nitromethane (14 mL) was added $Hg(CN)_2$ (126.3 mg, 0.5 mmol), and 7 mL of the solvent was distilled off at atmospheric pressure. The mixture was then cooled and stirred with powdered molecular sieve (4 Å, 150 mg) for 2 h. Bromide **27** (250 mg, 0.26 mmol) was then added and stirring was continued overnight. Conventional work-up and column chromatography (8:2 CH_2Cl_2–acetone) of the product yielded *O*-(2,4,6-tri-*O*-acetyl-3-*O*-methyl-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-[3,6-di-*O*-acetyl-α-D-glucopyranose 1,2-(2,3,4,2',3',4',6'-hepta-*O*-acetyl-α,α-trehalos-6-yl orthoacetate)] (**28**; 191 mg, 61.7%), $[\alpha]_D$ +63° (*c* 0.95, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): ^{1}H, δ 5.66 (d, 1 H, $J_{1c,2c}$ 3.2 Hz, H-1c), 3.425 (s, 3 H, OMe), 2.25–2.00 (m, 45 H, 15 OAc), 1.65 (s, 3 H, *endo*-Me of the orthoester); ^{13}C, δ 121.07 (quaternary carbon of orthoester moiety), 101.66, 100.94 (C-1D, C-1E), 96.78 (C-1C), 92.23, 92.13 (C-1A, C-1B), 81.27 (C-3E), 78.26 (C-3D), 76.86 (C-4C), 63.20 (C-6B), 62.06 (3 C, C-6C,6D,6E), 61.70 (C-6A).

Anal. Calc. for $C_{63}H_{86}O_{42}$: C, 49.94; H, 5.72. Found: C, 50.01; H, 5.76.

*O-(2,4,6-Tri-*O*-acetyl-3-*O*-methyl-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl)-(1↔1)-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside)* (**29**). — A solution of **28** (140 mg) in dichloromethane–chloroform (1:1; 3 mL) was stirred with powdered molecular sieves (4 Å) for 4 h.

One drop of $BF_3.Et_2O$ was then added, and the mixture was stirred for 4 days at room temperature, then diluted with dichloromethane (20 mL), filtered, and concentrated. Column chromatography of the residue gave **29** (55 mg, 39.3%), isolated as a foam, $[\alpha]_D$ +58° (*c* 0.95, chloroform).

Anal. Calc. for $C_{63}H_{86}O_{42}$: C, 49.94; H, 5.72. Found: C, 49.72; H, 5.77.

O-β-D-Glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1↔1)-α-D-glucopyranoside (**30**). — Compound **25** (0.17 g) was saponified by the Zemplén method. Column chromatography (1-butanol–methanol–water, 2:1:1) of the product gave **30** (72 mg, 81.2%), isolated as a glass, $[\alpha]_D$ +90° (*c* 1, water).

Anal. Calc. for $C_{18}H_{32}O_{16}$: C, 42.86; H, 6.39. Found: C, 42.79; H, 6.44.

O-β-D-Glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1↔1)-α-D-glucopyranoside (**2**). — Zemplén deacetylation of **26** (180 mg) and column chromatography (2:1:1 1-butanol–methanol–water) of the product gave amorphous **2** (77 mg, 80.5%), $[\alpha]_D$ +67° (*c* 0.5, water).

Anal. Calc. for $C_{24}H_{42}O_{21}$: C, 43.25; H, 6.35. Found: C, 43.32; H, 6.41.

O-(3-O-Methyl-β-D-glucopyranosyl)-(1→3)-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1↔1)-α-D-glucopyranoside (**1**). — Zemplén deacetylation of **29** (40 mg), as described above, gave amorphous **1** (20.2 mg, 90.8%), $[\alpha]_D$ +61° (*c* 0.8, water).

Anal. Calc. for $C_{31}H_{54}O_{26}$: C, 44.18; H, 6.46. Found: C, 44.30; H, 6.52.

ACKNOWLEDGMENT

We thank Professor Dr. P. Nánási for his interest.

REFERENCES

1 S. W. Hunter, T. Fujiwara, and P. J. Brennan, *J. Biol. Chem.*, 257 (1982) 15072–15078.
2 P. J. Brennan and M. B. Goren, *J. Biol. Chem.*, 254 (1979) 4205–4211.
3 S. Saadat and C. E. Ballou, *J. Biol. Chem.*, 258 (1983) 1813–1818.
4 S. W. Hunter, R. C. Murphy, K. Clay, M. B. Goren, and P. J. Brennan, *J. Biol. Chem.*, 258 (1983) 10481–10487.
5 W. Fischer and J. Krieglstein, *Hoppe-Seyler's Z. Physiol. Chem.*, 348 (1967) 1252–1255.
6 A. D. Elbein, *Adv. Carbohydr. Chem. Biochem.*, 30 (1974) 227–256.
7 G. G. Birch, *Adv. Carbohydr. Chem.*, 18 (1963) 201–225.
8 A. Klemer, E. Bühe, and R. Kutz, *Justus Liebigs Ann. Chem.*, 739 (1970) 185–193.
9 J. C. Sowden and A. Spriggs, *J. Am. Chem. Soc.*, 78 (1956) 2503–2505.
10 S. Koto, N. Morishima, and S. Zen, *Chem. Lett.*, (1976) 61–64.
11 R. U. Lemieux and H. F. Bauer, *Can. J. Chem.*, 32 (1954) 340–344.
12 G. J. F. Chittenden, *Carbohydr. Res.*, 9 (1969) 323–326.
13 A. A. Pavia, J. M. Pochemville, and S. N. Ung, *Carbohydr. Res.*, 79 (1980) 79–89.
14 J. Yoshimura, K. Hara, T. Sato, and H. Hashimoto, *Chem. Lett.*, (1983) 319–320.
15 A. Lipták and M. Gondor, *Acta Chim. Hung.*, 114 (1984) 309–321.
16 T. Fujiwara, S. W. Hunter, and P. J. Brennan, *Carbohydr. Res.*, 148 (1986) 287–298.
17 R. W. Jeanloz and D. A. Jeanloz, *J. Am. Chem. Soc.*, 79 (1957) 2579–2583.
18 B. Helferich and K. F. Wedemeyer, *Justus Liebigs Ann. Chem.*, 563 (1949) 139–145.
19 A. Lipták, Z. Szurmai, P. Nánási, and A. Neszmélyi, *Carbohydr. Res.*, 99 (1982) 13–21.
20 B. A. Dmitriev, A. V. Nikolaev, A. S. Shashkov, and N. K. Kochetkov, *Carbohydr. Res.*, 100 (1982) 195–206.

21 K. TAKEO, T. NAKAJI, AND K. SHINMITSU, *Carbohydr. Res.*, 133 (1984) 275–287.
22 H. PAULSEN AND R. LEBUHN, *Justus Liebigs Ann. Chem.*, (1983) 1047–1072.
23 K. TAKEO, K. OKUSHIO, K. FUKUYAMA, AND T. KUGE, *Carbohydr. Res.*, 121 (1983) 163–173.
24 P. J. GAREGG, T. IVERSEN, AND S. OSCARSON, *Carbohydr. Res.*, 50 (1976) c12–c14.
25 E. J. COREY AND J. W. SUGGS, *J. Org. Chem.*, 38 (1973) 3224.
26 P. A. GENT AND R. GIGG, *J. Chem. Soc., Chem. Commun.*, (1974) 277–278.
27 B. HELFERICH, *Adv. Carbohydr. Chem.*, 3 (1948) 79–111.
28 N. K. KOCHETKOV, A. J. KHORLIN, AND A. F. BOCHKOV, *Tetrahedron*, 23 (1967) 693–707.
29 A. F. BOCHKOV AND Y. V. VOZNYI, *Carbohydr. Res.*, 32 (1974) 1–8.
30 K. BOCK AND M. MELDAL, *Acta Chem. Scand., Ser. B*, 33 (1983) 775–783.
31 K. BOCK, C. PEDERSEN, AND H. PEDERSEN, *Adv. Carbohydr. Chem. Biochem.*, 42 (1984) 193–225.