Synthesis of the colitose determinant of *Escherichia coli* O111 and 3,6-di-*O*-(α-D-galactopyranosyl)-α-D-glucopyranoside[1]

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Received September 16, 1981

TOMMY IVERSEN and DAVID R. BUNDLE. Can. J. Chem. **60**, 299 (1982).

The syntheses of two branched trisaccharides, which constitute important elements of enterobacterial lipopolysaccharides are described. The common structural feature of each trisaccharide is α-D-glucopyranoside, upon which branching occurs at the *O*-3 and *O*-6 positions. Selective blocking of this hexoside at *O*-2 and *O*-4 by persistent blocking groups was accomplished by benzylation of 1,6-anhydro-β-D-glucopyranoside (**1**). Acetolysis of the product **2** afforded a mixture of anomeric triacetates **3** from which the corresponding 3,6-di-*O*-acetyl-2,4-*O*-benzyl-α-D-glucopyranosyl chloride (**4**) was prepared. The α and β 8-methoxycarbonyloctyl glycosides **5** and **6** were obtained in the ratio ~2:1, when **4** was reacted with 8-methoxycarbonyloctanol. Transesterification of the α-glycoside **5** gave the aglyconic intermediate **7** from which each trisaccharide could be obtained in a one step reaction. Thus glycosylation of **7** with tetra-*O*-benzyl-α-D-galactopyranosyl chloride (**8**) or 2,4-di-*O*-benzyl-3,6-dideoxy-α-L-*xylo*-hexopyranosyl chloride (**10**) gave the trisaccharides **11** and **13**. In both cases removal of blocking groups was achieved in a single hydrogenolysis step. Trisaccharide **12** represents a trisaccharide sequence present in the core saccharide of Salmonella lipopolysaccharides, while the colitose containing trisaccharide **14** is an essential element of the *E. coli* O111 *O*-antigen.

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On décrit la synthèse de deux trisaccharides ramifiés qui constituent des éléments importants des lipopolysaccharides entérobactériens. La caractéristique structurale commune à chaque trisaccharide est l'α-D-glucopyrannoside, sur lequel s'effectue les ramification en positions *O*-3 et *O*-6. On a pu protéger sélectivement cet hexoside en positions *O*-2 et *O*-4 à l'aide de groupes protecteurs très stables en benzylant l'anhydro-1,6 β-D-glucopyrannose (1). L'acélotyse du composé **2** conduit à un mélange de triacétates **3** anomères à partir duquel on a préparé le chlorure de di-*O*-acétyl-3,6 *O*-benzyl-2,4 α-D-glucopyrannosyle (**4**). On a obtenu les α- et β-glycosides de methoxycarbonyl-8 octyle, **5** et **6**, dans un rapport de 2:1 environ en faisant réagir le composé **4** avec le méthoxycarbonyl-8 octanol. La transestérification de l'α-glycoside conduit à l'intermédiaire aglyconique **7** à partir duquel on peut obtenir chaque trisaccharide en une seule étape. Ainsi la glycosilation du composé **7** par le chlorure de tétra-*O*-benzyl α-D-galactopyrannosyle (**8**) ou par le chlorure de di-*O*-benzyl-2,4 didéoxy-3,6 α-L-xylohexopyrannosyle (**10**) donne les trisaccharides **11** et **13**. Dans les deux cas, l'hydrolyse permet d'effectuer la déprotection en une seule étape. Le trisaccharide **12** représente une suite de trisaccharides présents dans le noyau saccharique des lipopolysaccharides du type Salmonella, tandis que le trisaccharide **14** contenant la colitose est un élément essentiel de l'antigène O de l'*E. Coli* O111.

[Traduit par le journal]

Introduction

Although antigenic determinants of *Salmonella* O-antigens containing the three 3,6-dideoxyhexoses paratose, abequose, and tyvelose have been synthesized (1–3), none of the colitose (optical antipode of abequose)-containing determinants have yet been prepared. Preliminary data indicate that colitose occurs in a "cross"-like structure as part of the repeating unit of the *E. coli* O111 O-antigen (4). Serological cross-reactions of *E. coli* O111, *Salmonella adelaide* O–35, and *Salmonella greenside* O–50 are related to the presence of colitose in the lipopolysaccharides (LPS) from each of these bacteria (5–7). In order to investigate further the dependence of these serological cross-reactions upon the colitose residues, the branched, colitose-containing trisaccharide, which is part of the *E. coli* O-antigen, was synthesized. The structurally related galactose-containing trisaccharide of *Salmonella* LPS core structures (8) was also synthesized. Since recent work has shown approximately 65% of LPS molecules to be incomplete with respect to addition of the O-chains (9, 10), the accessibility of such incomplete structures to core-specific antibodies is of renewed interest (11).

The synthesis of both trisaccharides was contingent upon an acceptable blocking strategy, which would provide an α-D-glucopyranoside with persistent blocking groups at *O*-2 and *O*-4, leaving *O*-3 and *O*-6 open for glycosylation. Branching at *O*-3 and *O*-6 of galacto- and manno-pyranosides occurs in blood-group substances (12) and glycoproteins (13). Compared with these hexoses, glucose lacks an axial–equatorial vicinal diol system that may be exploited to generate the required substitution pattern of blocking groups (14–16). A solution to this problem is described here, which facilitated the synthesis of two biologically important branched structures.

Results

The scheme envisaged for the synthesis of branched structures was based upon the requirement

[1]Issued as NRCC No. 19790.

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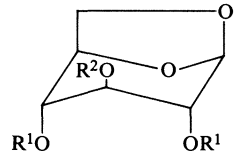
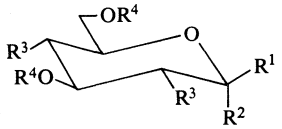
0008-4042/82/030299-05$01.00/0

for a 8-methoxycarbonyloctyl α-D-glycopyranoside bearing persistent blocking groups, preferably benzyl ethers, at *O*-2 and *O*-4. In order to accomplish this, methyl α-D-glucopyranoside and *tert*-butyl β-D-glucopyranoside were reacted with *tert*-butyldiphenylsilyl chloride in DMF (17). Whereas the methyl glycoside gave predominantly the 2,6-disubstituted glucoside, *tert*-butyl β-D-glucopyranoside (18) gave the desired 3,6-disilyl ether. Unfortunately this product proved to be resistant to benzylation conditions, which have been reported to be compatible with the integrity of such silyl ethers (17).

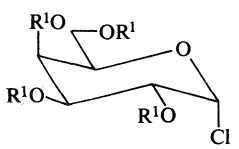
A suitable derivative, 2,4-di-*O*-benzyl-1,6-anhydro-β-D-glucopyranose, has been reported in the literature (19–21). Benzylation of 1,6-anhydro-β-D-glucopyranose (**1**) with benzyl bromide and barium oxide in DMF gave **2** in 95% yield compared with the previously reported yield of 21%. Acetolysis of **2** gave an anomeric mixture of the triacetates **3** in 77% yield. This mixture was converted cleanly and quantitatively to 3,6-di-*O*-acetyl-2,4-di-*O*-benzyl-α-D-glucopyranosyl chloride (**4**), which was used without purification for the glycosylation of 8-methoxycarbonyloctanol. The desired α-glycoside **5** was obtained together with the β-anomer **6** in the ratio ~2:1. Transesterification of **5** gave the selectively blocked α-glucopyranoside **7**, which was used for the one-step syntheses of the branched trisaccharides.



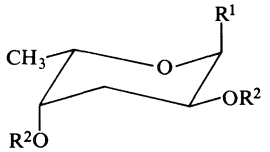
1 $R^1 = R^2 = H$
2 R^1 = Bzl, $R^2 = H$



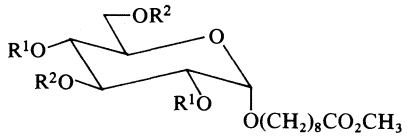
3 R^1/R^2 = Ac/H, R^3 = OBzl, R^4 = Ac
4 $R^1 = H$, R^2 = Cl, R^3 = OBzl, R^4 = Ac
5 $R^1 = H$, $R^2 = O(CH_2)_8CO_2CH_3$, R^3 = OBzl, R^4 = Ac
6 $R^1 = O(CH_2)_8CO_2CH_3$, $R^2 = H$, R^3 = OBzl, R^4 = Ac
7 $R^1 = R^4 = H$, $R^2 = O(CH_2)_8CO_2CH_3$, R^3 = OBzl



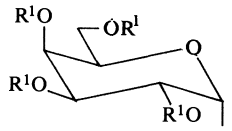
8 R^1 = Bzl



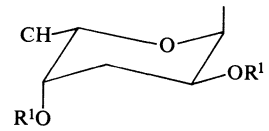
9 $R^1 = OCH_3$, R^2 = Bzl
10 R^1 = Cl, R^2 = Bzl



11 R^1 = Bzl, R^2 =
12 $R^1 = H$, R^2 =



13 R^1 = Bzl, R^2 =
14 $R^1 = H$, R^2 =



Silver trifluoromethanesulphonate (triflate)-promoted glycosylation of **7** by tetra-*O*-benzyl-α-D-galactopyranosyl chloride (**8**) gave the corresponding trisaccharide **11** in 57% yield. Carbon-13 nmr revealed the presence of three anomeric carbon atoms with chemical shifts characteristic of α-anomers.

Methyl 3,6-dideoxy-α-L-*xylo*-hexopyranoside (22) was benzylated to give the benzylated glycoside **9**. Conversion of this derivative to the glycosyl chloride **10** was accomplished by reaction of the methyl glycoside **9** with dichloromethyl methyl ether in the presence of zinc chloride (23). The chlorosugar **10** was used immediately without purification. Glycosylation of the aglyconic component **7** by **10** under conditions of standard halide-ion catalysis (24) was unsuccessful. Under less conventional conditions the glycosyl chloride **10** reacted with the selectively blocked glucoside **7** in acetonitrile solution to give the required trisaccharide **13** in 53% yield.

The blocked trisaccharides **11** and **13** were hydrogenated to give the deblocked trisaccharides **12** and **14**. Proton and carbon-13 nmr chemical shifts confirm the α-configuration of the glycosidic linkages.

Discussion

The selective protection of D-glucose residues is made difficult by the equatorial relationship of the secondary hydroxyl groups of the $^4C_1$ (D) conformer. These difficulties may be circumvented for some purposes by the use of 1,6-anhydro-β-D-glucopyranose (**1**) (20). For the purpose of generating 3,6-disubstituted glucopyranose residues, benzylation of **1** to give the 2,4-di-*O*-benzyl-glucopyranose **2** offers the most direct route (19–21), although the published yield is low (19). By comparison, an alternate route to a 2,4-di-*O*-benzyl-D-glucopyranoside was recently reported (24). Modification of the benzylation conditions of **1** gave **2** in

good yield. Acetolysis followed by conversion to the glycosyl chloride **4** provides a rapid route via glycosylation of 8-methoxycarbonyloctanol to the desired intermediate α-glycosides **5** and **7**. The yields achieved compare favourably with previous work (19).

Methyl 2,4-di-*O*-benzyl-3,6-dideoxy-α-L-*xylo*-hexopyranoside (**9**) was efficiently converted to the corresponding glycosyl chloride **10** by dichloromethyl methyl ether with zinc chloride as the Lewis acid (23). The 3,6-dideoxyglycosyl halides are among the most labile glycosyl halides. These reaction conditions are, therefore, compatible with benzyl ether blocking groups and well suited to the lability of the product.

Throughout this study glycosyl chlorides were employed, a choice based on several considerations. The chlorides are more stable than the glycosyl bromide analogues and the reduced reactivity can be compensated for by judicious choice of promoter, according to the scheme of Paulsen and Kolář (25). Thus tetra-*O*-benzyl-α-D-galactopyranosyl chloride (**8**) readily formed α-glycosidic bonds at *O*-6 and *O*-3 with silver triflate as promoter (26).

When the glycosyl chloride **10** was reacted with 8-methoxycarbonyloctyl 2,4-di-*O*-benzyl-α-D-glucopyranoside (**7**) under conditions of halide ion catalysis, the reaction mixture contained many components from which only decomposition products of **10** and **7** could be isolated. In order to suppress side reactions which could result from the electrophilic nature of reaction intermediates (27), acetonitrile was employed as the solvent for the halide-ion catalysed reaction of **10** with **7**. The solvating action of acetonitrile served to stabilize the glycosyl chloride, the presence of which was evident from tlc examination of the reaction mixture over a five-day period. In this way an efficient yield of the trisaccharide **12** was secured.

The deblocked glycosides showed nmr parameters in agreement with the structures. The ^{1}H nmr shift and ^3J coupling constant data for the three anomeric protons are consistent only with the presence of α-linkages in both trisaccharides **12** and **14**. The ^{13}C nmr shifts for the anomeric carbon atoms further corroborate this conclusion.

Binding of antibody, raised against *E. coli* O111 or *Salmonella adelaide* whole cell vaccines, to the respective cell wall lipopolysaccharides was inhibited by methyl 3,6-dideoxy-α-L-*xylo*-hexopyranoside and trisaccharide **14**. In all cases the trisaccharide was at least 50–100 times more effective than the methyl α-glycoside of colitose. These results reflect the crucial spacial disposition of the two colitose residues for antibody binding. Moreover, since trisaccharide **14** inhibits the homologous *S. adelaide* system, both *S. adelaide* and *E. coli* O111 likely possess similar O-antigen structures. This conclusion based on serology is supported by independent structural studies.[3]

Experimental

The general methods and materials employed in this work are similar to those described in other papers from this laboratory (22, 28). Carbon 13 and ^{1}H nmr spectra were recorded at 20 and 79.9 MHz respectively. Proton chemical shifts are expressed relative to 1% tetramethylsilane (TMS) for deuteriochloroform solutions and, in the case of deuterium oxide, relative to sodium 3-trimethyl-silylpropionate-2,2,3,3-d_4 (TSP). Carbon-13 shifts are expressed relative to internal and external TMS for deuteriochloroform and deuterium oxide solutions. Medium pressure column chromatography was used for most separations and was conducted according to our published method (29).

*1,6-Anhydro-2,4-di-*O*-benzyl-β-*D*-glucopyranose (**2**)*

A mixture of 1,6-anhydro-β-D-glucopyranose (30) (**1**) (2.0 g, 12 mmol), benzyl bromide (7.5 mL), and barium oxide (7.5 g) in dimethylformamide (50 mL) was stirred at 60°C for 3 h, followed by addition of methanol (25 mL) to destroy excess benzyl bromide. After 30 min the reaction mixture was diluted with chloroform, filtered, and washed with water. Evaporation of the solvents left a syrup that was purified on a silica gel column (Skellysolve B – ethyl acetate 2:1) to give 4.0 g (95%) of **2**: $[\alpha]_D$ −28.8° (*c* 1.0, chloroform), mp 104.5–105°C (recryst. Skellysolve B – ethyl acetate) (lit. (19) $[\alpha]_D$ −28.5°; mp 106.5–107°C); ^{1}H nmr ($CDCl_3$) δ: 7.34 (bs, 10H, aromatics), 5.44 (s, 1H, H-1). *Anal.* calcd. for $C_{20}H_{22}O_5$: C 70.16, H 6.48; found: C 70.04, H 6.54.

*1,3,6-Tri-*O*-acetyl-2,4-di-*O*-benzyl-*D*-glucopyranose (**3**)*

Compound **2** (3.0 g, 8.8 mmol) was dissolved in acetic anhydride (50 mL) and 3 drops of concentrated sulfuric acid were added to the stirred reaction mixture. After 30 min the solution was diluted with chloroform, washed with aqueous sodium bicarbonate and water, and evaporated. The resulting syrup was purified on a silica-gel column (Skellysolve B – ethyl acetate 2:1) to give 3.3 g (79%) of **3** as an anomeric mixture; α/β ~3; $[\alpha]_D$ +80.7° (*c* 2.0, chloroform); ^{1}H nmr ($CDCl_3$) δ: 7.27 (bs, 10H, aromatics), 6.32 (d, $J_{1,2}$ = 3.6 Hz, H-1α), 5.45 (d, $J_{1,2}$ = 9.7 Hz, H-1β); ^{13}C nmr ($CDCl_3$) δ_c: 93.8 (C-1β), 89.2 (C-1α).

*3,6-Di-*O*-acetyl-2,4-di-*O*-benzyl-α-*D*-glucopyranosyl chloride (**4**)*

Dichloromethyl methyl ether (7.5 mL) was added to a solution of **3** (2.6 g, 5.3 mmol) and zinc chloride (~100 mg) in dichloromethane (50 mL). The reaction mixture was stirred for 1.5 h, filtered, evaporated, and dried under vacuum to give 2.5 g (~100%) of the syrupy chlorosugar **4**. Carbon-13 nmr and ^{1}H nmr indicated the syrup to be at least 95% pure and it was used in the glycosidation step without purification; ^{1}H nmr ($CDCl_3$) δ: 6.01 (d, 1-H, $J_{1,2}$ = 3.9 Hz, H-1), 5.59 (t, 1H, $J_{2,3} \approx J_{3,4}$ = 12 Hz, H-3), 2.07 (s, 3H, OAc), 2.00 (s, 3H, OAc); ^{13}C nmr ($CDCl_3$) δ_c: 92.14 (C-1).

*8-Methoxycarbonyloctyl 3,6-di-*O*-acetyl-2,4-di-*O*-benzyl-α- (**5**) and -β-*D*-glucopyranoside (**6**)*

The chlorosugar **4** (2.5 g, 5.3 mmol) dissolved in dichloromethane (5 mL) was added to a stirred mixture of 8-methoxycarbonyloctanol (31) (1.3 g, 6.9 mmol), mercuric cyanide (2.7 g,

[3]L. Kenne, private communication.

11 mmol), and mercuric bromide (3.7 g, 10 mmol) in dichloromethane (10 mL) at −70°C. The reaction was allowed to warm to room temperature and stirred overnight. Filtration and evaporation gave a syrupy residue that was purified on a silica-gel column (Skellysolve B – ethyl acetate 3:1) to give 1.6 g (49%) of **5**; $[\alpha]_D$ +68.5° (*c* 1.4, chloroform); R_f = 0.33 (solvent same as above); ^{1}H nmr ($CDCl_3$) δ: 7.32 (bs, 10H, aromatics), 5.56 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.4 Hz, H-3), 3.66 (s, 3H, OMe), 2.09 (s, 3H, AcO), 2.00 (s, 3H, AcO), 1.10–1.80 (m, 12H, —$(CH_2)_6$—); ^{13}C nmr ($CDCl_3$) δ_c: 96.6 (C-1); and 0.9 g (28%) of **6**; $[\alpha]_D$ +33.5° (*c* 1.2, chloroform); R_f = 0.35 (solvent same as above); ^{1}H nmr ($CDCl_3$) δ: 9.27 (bs, 10H, aromatics), 3.66 (s, 3H, OMe), 2.04 (s, 3H, AcO), 1.88 (s, 3H, AcO), 1.80–1.10 (m, 12H, —$(CH_2)_6$—); ^{13}C nmr ($CDCl_3$) δ_c: 103.5 (C-1).

*8-Methoxycarbonyloctyl 2,4-di-*O*-benzyl-α-*D*-glucopyranoside (7)*

Compound **5** (2.3 g, 3.7 mmol) in methanol (50 mL) containing a catalytic amount of sodium methoxide was left overnight. The syrup, obtained after removal of sodium ions with Rexyn 101 (H^+) resin, filtration, and evaporation, was purified on a silica-gel column (Skellysolve B – ethyl acetate 2:1) to give 1.6 g (83%) of **7**; $[\alpha]_D$ 79.6° (*c* 1.1, chloroform); ^{1}H nmr ($CDCl_3$) δ: 7.34 (bs, 10H, aromatics), 3.65 (s, 3H, OMe), 2.0–1.0 (m, 12H, —$(CH_2)_6$—); ^{13}C nmr ($CDCl_3$) δ_c: 96.5 (C-1). *Anal.* calcd. for $C_{30}H_{42}O_8$: C 67.90, H 7.98; found: C 67.70, H 7.88.

*8-Methoxycarbonyloctyl 2,4-di-*O*-benzyl-3,6-di-*O*-[tetra-*O*-benzyl-α-*D*-galactopyranosyl]-α-*D*-glucopyranoside (11)*

A solution of tetra-*O*-benzyl-α-D-galactopyranosyl chloride (**8**) (30) (2.0 g, 3.5 mmol) in dichloromethane (10 mL) was added to a stirred mixture of compound **7** (0.40 g, 0.8 mmol), silver triflate (1.5 g, 5.8 mmol), *N*,*N*-tetramethylurea (3 mL), and molecular sieve 4 Å in dichloromethane (15 mL) at −40°C. The reaction mixture was allowed to warm to room temperature and stirred overnight. Filtration followed by evaporation left a syrup that was purified on a silica-gel column (toluene – ethyl acetate 15:1) to give 0.68 g (57%) of **8**; $[\alpha]_D$ +53.1° (*c* 1.4, chloroform); ^{13}C nmr ($CDCl_3$) δ_c: 98.3, 97.9, 96.5 (C-1″, C-1′, and C-1).

*Methyl 2,4-di-*O*-benzyl-3,6-dideoxy-α-*L*-xylo-hexopyranoside (9)*

A solution of methyl 3,6-dideoxy-α-L-*xylo*-hexopyranoside (22) (5.9 g, 36 mmol) containing sodium hydride (3.5 g, 15 mmol) in dry dimethylformamide (100 mL) was stirred at room temperature for 30 min. Benzyl bromide (20 g) in dimethylformamide (50 mL) was then slowly added to the stirred mixture and the reaction was left for 2 h at room temperature. Excess benzyl bromide was destroyed by the addition of methanol (25 mL). The reaction mixture was diluted with dichloromethane, washed with water, and evaporated to a syrup that was purified on a silica-gel column (Skellysolve B – ethyl acetate 4:1) to give 9.5 g (76%); $[\alpha]_D$ −19.8° (*c* 1.7, chloroform); ^{1}H nmr ($CDCl_3$) δ: 3.90 (s, 3H, OMe), 2.20–1.70 (m, 2H, H-4), 1.28 (d, 3H, $J_{5,6}$ = 6 Hz, H-6); ^{13}C nmr ($CDCl_3$) δ_c: 98.0 (C-1), 27.9 (C-3), 16.6 (C-6). *Anal.* calcd. for $C_{21}H_{26}O_4$: C 73.66, H 7.65; found: C 73.61, H 7.66.

*2,4-Di-*O*-benzyl-3,6-dideoxy-α-*L*-xylo-hexopyranosyl chloride (10)*

Anhydrous zinc chloride (~50 mg) was added to a solution of **9** (0.53 g, 1.5 mmol) in dichloromethyl methyl ether (5 mL). The reaction mixture was stirred at room temperature for 1 h, filtered through glass wool, evaporated, and dried under high vacuum to give 0.53 g (~ 100%) of the syrupy chlorosugar **10**, which was immediately used in the glycosidation step; ^{1}H nmr ($CDCl_3$) δ: 6.26 (d, 1H, $J_{1,2} \approx$ 2 Hz, H-1), 2.35–1.95 (m, 2H, H-3), 1.26 (d, 3H, $J_{5,6} \approx$ 6 Hz, H-6); ^{13}C nmr ($CDCl_3$) δ_c: 95.8 (C-1), 28.1 (C-3), 16.3 (C-6).

*8-Methoxycarbonyloctyl-2,4-di-*O*-benzyl-3,6-di-*O*-[2,4-di-*O*-benzyl-3,6-dideoxy-α-*L*-xylo-hexopyranosyl]-α-*D*-glucopyranoside (13)*

The chlorosugar **10** (0.53 g, 1.5 mmol) from the above preparation, in acetonitrile (5 mL), was added to a stirred mixture of compound **7** (0.35 g, 0.7 mmol), tetraethylammonium chloride (0.66 g, 4 mmol), diisopropylethylamine (0.25 g, 1.9 mmol), and molecular sieve 4 Å in acetonitrile (10 mL) at −40°C. The reaction mixture was allowed to reach room temperature and was stirred for 10 days, after which time the mixture was filtered and evaporated to a syrupy residue that was separated on a silica-gel column (Skellysolve B – ethyl acetate 4:1) to give 0.40 g (53%) of compound **11**; $[\alpha]_D$ −8.5° (*c* 0.7, chloroform); ^{13}C nmr ($CDCl_3$) δ_c: 96.9, 96.5, 95.9 (C-1″, C-1′, and C-1).

*8-Methoxycarbonyloctyl 3,6-di-*O*-[α-*D*-galactopyranosyl]-α-*D*-glucopyranoside (12)*

The trisaccharide **8** (0.67 g, 0.4 mmol) was dissolved in ethanol – acetic acid (1:1, 80 mL) and hydrogenolyzed for 18 h at 70 psi over 10% palladium on charcoal. Filtration and evaporation of solvents left a syrup that was purified on a silica-gel column (ethyl acetate – methanol–water 7:2:1) to give 0.18 g (63%) of the product **12**. The ^{13}C nmr of **12** showed the presence of small amounts of disaccharide impurities and an analytically pure sample was obtained by acetylation (acetic anhydride – pyridine) and chromatography on a silica-gel column (Skellysolve B – ethyl acetate 4:1) followed by deacetylation (sodium methoxide – methanol); $[\alpha]_D$ +79.2° (*c* 0.9, in water); ^{1}H nmr (D_2O, 90°C) δ: 5.96 (s, 1-H, H-1″), 4.97 (dd, 2-H, $J_{1,2} \approx$ 1.4, $J_{1,2}$ = 3.7 Hz, H-1′, H-1), 2.4 (t, —CH_2CO—), 2.0–1.1 (m, 12H, —$(CH_2)_6$—); ^{13}C nmr (D_2O) δ_c: 100.5, 99.6, and 99.3 (C-1″, C-1′, and C-1). *Anal.* calcd. for $C_{28}H_{50}O_{18}$: C 49.85, H 7.47; found: C 49.61, H 7.58.

*8-Methoxycarbonyloctyl 3,6-di-*O*-[3,6-dideoxy-α-*L*-xylo-hexopyranosyl]-α-*D*-glucopyranoside (14)*

The trisaccharide (**11**) (180 mg, 0.2 mmol) was dissolved in ethanol (30 mL) and hydrogenolyzed for a week at 70 psi over 10% palladium on charcoal. Filtration and evaporation of the solvent left a syrup that was purified on a silica-gel column (ethyl acetate – methanol–water 85:10:5) to give 80 mg (84%) of the product **13** $[\alpha]_D$ −17.7° (*c* 0.6, H_2O); ^{1}H nmr (D_2O, 90°C) δ: 5.16 (d, 1-H, $J_{1',2'}$ = 3.6 Hz, H-1′), 4.88 (dd, 2H, $J_{1,2}$ = 3.0 Hz, $J_{1,2}$ = 4.2 Hz, H-1, H-1″); ^{13}C nmr (D_2O) δ_c: 100.5, 99.7, 99.5 (C-1″, C-1′, and C-1), 16.7 (C-6″), 16.6 (C-6′). *Anal.* calcd. for $C_{28}H_{50}O_{14}$: C 55.07, H 8.25; found: C 54.91, H 8.42.

Acknowledgements

We wish to thank Dr. Klaus Bock for valuable discussions and for a generous gift of 1,6-anhydro-β-D-glucopyranose. In addition, the expert assistance of Mr. H. Seguin for microanalyses and the technical assistance of Mr. J. W. L. C. Christ are gratefully acknowledged.

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