Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 338 (2003) 1033–1037
CARBOHYDRATE RESEARCH
www.elsevier.com/locate/carres

First synthesis of β-D-Gal*f*-(1→3)-D-Gal*p*—the repeating unit of the backbone structure of the O-antigenic polysaccharide present in the lipopolysaccharide (LPS) of the genus *Klebsiella*

Hairong Wang,[b] Guohua Zhang,[a] Jun Ning[a,*]

[a]*Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China*
[b]*Department of Chemistry, Tsinghua University, Beijing 100084, China*

Received 11 November 2002; accepted 12 February 2003

Abstract

β-D-Galactofuranosyl-(1→3)-D-galactopyranose (**1**), the repeating unit of the backbone structure of the O-antigenic polysaccharide present in the lipopolysaccharide (LPS) of the genus *Klebsiella*, has been efficiently synthesized using 1,2:5,6-di-*O*-isopropylidine-α-D-galactofuranose (**3**) as the glycosyl acceptor and 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl trichloroacetimidate (**6**) as the glycosyl donor with TMSOTf as catalyst by the well-known Schmidt glycosylation method. The preparation of **3** was improved by increasing the ratio of DMF to acetone and employing a solid-supported catalyst.

Keywords: Oligosaccharides; Galactofuranose; Synthesis

1. Introduction

Although unknown in human biology, polysaccharides containing galactofuranosyl residues are important constituents of glycoconjugates from many lower organisms including bacteria,[1] protozoa,[2] and fungi.[3] Glycans containing Gal*f* moieties are generally found in the extracellular glycocalyx (cell-wall complex) and consequently play critical roles in the survival and pathogenicity of these microorganisms. A well-established method for the treatment of bacterial disease is the use of antibiotics that act by inhibiting cell-wall biosynthesis.[4] Given the xenobiotic nature of Gal*f*-containing polysaccharides to humans, the biosynthetic pathways leading to their formation are particularly attractive targets for drug action. However, the processes by which these polysaccharides are assembled in nature are not well understood, and much additional research in this area is needed before this goal can be achieved.

While a number of microorganisms produce polysaccharides containing the Gal*f* residue, among the most important are the *Klebsiella*. This genus of bacteria contains a number of opportunistic pathogens that cause serious infections, including pneumonia, bacteremia, and urinary-tract infections.[5] A major component of the outer membrane of many bacteria is lipopolysaccharide (LPS) which comprises a hydrophobic lipid A portion, a core oligosaccharide, and an O-antigenic polysaccharide. The O-polysaccharide varies in structure from strain to strain, giving rise to unique antigenic epitopes. In the genus *Klebsiella*, there exists a family of structurally related galactose-containing O-polysaccharides. These are based on a backbone structure consisting of a disaccharide repeating unit →3)-β-D-Gal*f*-(1→3)-α-D-Gal*p*-(1→ known as D-galactan I.[6] Synthesis of the galactofuranosyl residue containing oligosaccharides present in the cell wall of bacteria is very important. On one hand, these oligosaccharides could be valuable models for immunoassay studies; on the other hand, they can be used as synthetic substrates for fundamental biochemical studies that may lead to the isolation and purification of the appropriate biosynthetic enzymes. This, together with

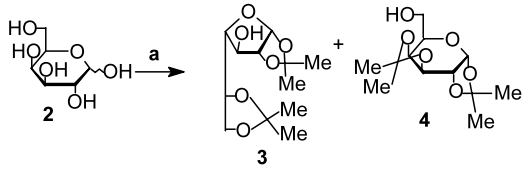
* Corresponding author. Fax: +86-10-62923563
E-mail address: jning@mail.rcees.ac.cn (J. Ning).

0008-6215/03/$ - see front matter
doi:10.1016/S0008-6215(03)00076-4

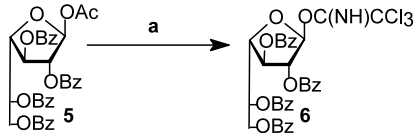
the fact that the synthesis of β-D-Gal*f*-(1→3)-D-Gal*p* (**1**) was not previously reported, prompted us to synthesize the disaccharide.

2. Results and discussion

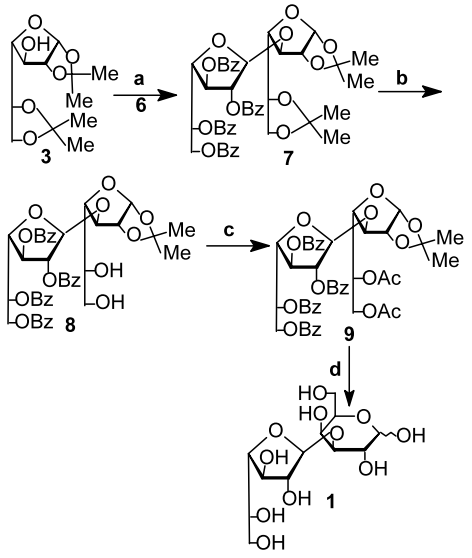
In our synthesis, 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose (**3**) is a key starting material. So far, 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose (**3**) has found only limited use in synthetic carbohydrate chemistry because it is not easily accessible. For example, **3** has been obtained from D-glucose derivatives in three- or six steps,[7] or from D-galactose in a maximum 22% yield.[8] Recently, Rauter's group found that by using Zeolite HY as the catalyst, the furanose diketal **3** was formed in 40% yield, together with the pyranose diketal **4**, obtained only in 20% yield, which significantly improved the preparation of **3**.[9] Some reports showed that high temperature is a factor known to favor furanose formation in solutions of reducing sugars.[10] Inspired by this, we increased the ratio of *N*,*N*-dimethylformamide (DMF) to acetone to 2:1 in our synthesis of **3**, attempting to increase the reaction reflux temperature in order to obtain more furanose diketal **3**. To simplify the purification procedure, an anhydrous Dowex 50 [H^+] resin was used as the solid-supported catalyst (Scheme 1). Furthermore, 4 Å molecular sieves were used in our reaction as the water scavenger in order to increase the yield. As a result of our reaction conditions, the ratio of 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose to 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose in the reaction product can reach more than 4:1, and the desired 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose can be easily isolated by flash chromatography, followed by crystallisation in 50% yield.[11]



Scheme 1. Reagents and conditions: (a) 2:1 DMF–acetone, Dowex 50 [H^+] (dry), 4 Å MS, reflux, 48 h, 50%.



Scheme 2. Reagents and conditions: (a) (i) 3:1 THF–CH_3OH, 1.5 N NH_3, rt, 3 h; (ii) CH_2Cl_2, CCl_3CN (2.0 equiv), K_2CO_3 (2.0 equiv), rt, 12 h, 82% (over two steps).



Scheme 3. Reagents and conditions: (a) TMSOTf (cat.), CH_2Cl_2, MS 4 Å Powder, rt, 3 h, 85%. (b) (i) 90% HOAc, 40 °C, 4 h; (ii) AcO_2, pyridine, rt, 2 h, 94% (over two steps). (c) 10:1 $HCCl_3$–CF_3COOH, rt, 2 h, 90%. (d) ammonia-saturated CH_3OH, rt, 72 h, 96%.

The glycosyl donor **6** was synthesized from 1-*O*-acetyl-2,3,5,6-tetra-*O*-benzoyl-D-galactofuranose (**5**)[12] by selective 1-O-deacetylation with ammonia in THF–CH_3OH, and then trichloroacetimidation with trichloroacetonitrile in the presence of K_2CO_3 (Scheme 2). The structure of **6** was confirmed by ^1H NMR spectral analysis. The $J_{1,2}$ is small (0–2 Hz) for the β anomers[13] and larger (4–5 Hz) for the α anomers.[13]

Condensation[14] of **3** and **6** with TMSOTf as catalyst and 4 Å molecular sieves in CH_2Cl_2 at room temperature smoothly afforded the disaccharide **7** in an excellent yield (85%) (Scheme 3). The structure of **7** was unambiguously confirmed by ^1H and ^{13}C NMR data. The two anomeric proton signals in the ^1H NMR spectrum of **7** were located at 5.91 ppm as a doublet with $J_{1,2} = 3.9$ Hz, and at 5.44 ppm as a singlet, respectively, whereas the ^{13}C NMR spectrum revealed[15] anomeric carbons at 105.1 and 104.8 ppm. Removal of the 1,2:5,6-di-*O*-isopropylidene group of **7** in 10:1 $CHCl_3$–CF_3COOH (v/v) at room temperature did not give satisfactory results because the reaction products are too complex. However, selective removal of the 5,6-*O*-isopropylidene group of **7** with 90% HOAc at 40 °C, followed by acetylation using Ac_2O in pyridine and subsequent 1,2-O-deisopropylidenation with 10:1 $CHCl_3$–CF_3COOH (v/v) at room temperature, smoothly gave the required compound **9** in 84.6% yield (over three steps). Deprotection of **9** with an ammonia-saturated CH_3OH gave the desired disaccharide **1** in 96% yield. The ^{13}C NMR spectrum revealed anomeric carbons at 109.01 (C-1 for Gal*f*), 105.98 (C-1 for β-Gal*p*) and 96.0 (C-1 for α-Gal*p*) ppm, and thin-layer chromatography (TLC) (methanol) analysis showed two spots, indicating that compound **1** was a mixture of

α and β isomers. O-Deacylation of blocked oligosaccharides with methanolic sodium methoxide sometimes could be complicated by a β-elimination reaction typical of 3-substituted reducing aldoses;[16,17] however, this problem can be avoided by using ammonia-saturated CH_3OH.[17,18]

In conclusion, a highly concise synthesis of the repeating unit disaccharide **1** present in LPS of the genus *Klebsiella* was achieved. The simple and efficient preparation of the galactofuranose-containing oligosaccharide present in the bacterial outer membrane should promote studies on bacteria diseases.

3. Experimental

3.1. General methods

Optical rotations were determined at 20 °C with a Perkin–Elmer Model 241-Mc automatic polarimeter. Melting points (mp) were determined with a 'Mel-Temp' apparatus. ^1H and ^{13}C NMR spectra were recorded with Bruker ARX 400 spectrometers (400 MHz for ^1H, 100 MHz for ^{13}C) for solutions in $CDCl_3$ or CD_3OD as indicated. Chemical shifts are given in ppm downfield from internal Me_4Si. TLC was performed on silica gel HF_{254} with detection by charring with 30% (v/v) H_2SO_4 in MeOH or in some cases by a UV detector. Electrospray-ionization mass spectra were recorded on an Autospec instrument. Column chromatography was conducted by elution of a column (16×240, 18×300, 35×400 mm) of silica gel (100–200 mesh) with EtOAc–petroleum ether (60–90 °C) as the eluent. Solutions were concentrated at < 60 °C under reduced pressure.

3.2. Preparation of 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose (3)

A solution of D-galactose (**2**) (10 g, 55.56 mmol) in dry and hot *N*,*N*-dimethylformamide (400 mL) was added to stirred acetone (200 mL), which contained dry Dowex 50 [H^+] resin (15 g) and 4 Å molecular sieves (8 g). The reaction mixture was stirred under reflux for 48 h. Solid material was filtered off, and the solution was concentrated to a syrup under vacuum. TLC (3:1 petroleum ether–EtOAc) showed that the ratio of 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose to 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose in the resultant residue was $> 4:1$. After the residue was decolorized and, in some degree, purified by subjection to a flash chromatography with 3:1 petroleum ether–EtOAC as eluent, compound **3** (7.2 g, 50%) was crystallized from petroleum ether–EtOAc as white crystals: mp 96.5–98 °C; (Lit.[8] 97–98 °C), $[\alpha]_D$ −33.8° (*c* 0.8, CH_3OH) (Lit.[8] −34°).

3.3. 2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl trichloroacetimidate (6)

A solution of 1-*O*-acetyl-2,3,5,6-tetra-*O*-benzoyl-D-galactofuranose (**5**)[12] (3.6 g, 5.64 mmol) in 1.5 N NH_3 solution of 3:1 THF–CH_3OH (80 mL) was kept at room temperature (rt) for 3 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) showed that the reaction was complete and the solution was concentrated. The resultant residue without purification was dissolved in dry CH_2Cl_2 (50 mL), and then CCl_3CN (2.6 mL, 12.4 mmol) and anhydrous K_2CO_3 (3 g, 21.8 mmol) were added. The reaction mixture was stirred at rt for 12 h, and solid material was filtered off. Concentration of the filtrate, followed by purification on a silica-gel column with 3:1 petroleum ether–EtOAc as eluent, gave the monosaccharide donor **6** (3.42 g, 82% over the two steps): $[\alpha]_D$ +28.3° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.72 (s, 1 H, OC(N*H*)CCl_3), 8.09–7.26 (m, 20 H, 4 Ph*H*), 6.70 (s, 1 H, H-1), 6.13 (m, 1 H, H-5), 5.78 (d, 1 H, *J* 4.3 Hz, H-3), 5.75 (s, 1 H, H-2), 4.86 (t, 1 H, *J* 4.3 Hz, H-4), 4.78–4.75 (m, 2 H, H-6a, 6b). Anal. Calcd for $C_{36}H_{28}Cl_3NO_{10}$: C, 58.35; H, 3.81. Found: C, 58.56; H, 3.74.

3.4. 2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose (7)

A solution of **3** (1.36 g, 5.25 mmol) and **6** (3.89 g, 5.25 mmol) in dry CH_2Cl_2 (50 mL) was stirred with activated 4 Å molecular sieves (1 g) at rt under an atmosphere of nitrogen for 20 min. To the solution was added MeSiOTf (12 μL). The reaction mixture was stirred for 2 h, at the end of which time TLC (2.5:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was neutralized with Et_3N and filtered, and the filtrate was concentrated. The resultant residue was subjected to the column chromatography with 2:1 petroleum ether–EtOAc as eluent to afford the disaccharide **7** (3.74 g, 85%): $[\alpha]_D$ −26.5° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.08–7.26 (m, 20 H, 4 Ph*H*), 6.05 (m, 1 H, H-5′), 5.92 (d, 1 H, *J* 3.9 Hz, H-1), 5.71 (d, 1 H, *J* 4.5 Hz, H-3′), 5.50 (s, 1 H, H-1′), 5.44 (s, 1 H, H-2′), 4.81–4.66 (m, 4 H, H-2, 4′, 6′a, 6′b), 4.33 (m, 1 H, H-5), 4.20 (d, 1 H, *J* 4.5 Hz, H-3), 4.04–3.96 (m, 2 H, H-4, 6a), 3.86 (t, 1 H, *J* 7.5 Hz, H-6b), 1.55, 1.41, 1.36, 1.34 (4 s, 12 H, 2 $C(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.9, 165.6, 165.5, 165.4 (4 *C*OPh), 113.9, 109.9 (2 $C(CH_3)_2$), 105.1, 104.8 (2 C-1), 85.1, 84.2, 82.3, 82.0, 80.2, 77.1, 75.9, 70.3, 65.6, 63.3, 27.4, 26.7, 26.5, 25.3 (2 $C(CH_3)_2$). Anal. Calcd for $C_{46}H_{46}O_{15}$: C, 65.86; H, 5.53. Found: C, 65.66; H, 5.47.

3.5. 2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl-(1 → 3)-5,6-di-*O*-acetyl-1,2-*O*-isopropylidene-α-D-galactopyranose (8)

Compound **7** (3.8 g, 4.53 mmol) was dissolved in 90% HOAc (100 mL). The mixture was kept at 40 °C for 4 h, and then concentrated under reduced pressure. The resulting residue was treated with Ac_2O and Py at rt for 2 h and then purified by flash chromatography (1:1 petroleum ether–EtOAc) to give **8** (3.76 g, 94% over two steps): $[\alpha]_D$ −12.2° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.10–7.23 (m, 20 H, 4 Ph*H*), 6.15 (m, 1 H, H-5′), 5.87 (d, 1 H, *J* 4.1 Hz, H-1), 5.64 (d, 1 H, *J* 5.0 Hz, H-3′), 5.49 (s, 1 H, H-1′), 5.42 (s, 1 H, H-2′), 5.42 (m, 1 H, H-5), 4.83 (dd, 1 H, *J* 3.4, 12 Hz, H-6a′), 4.77–4.72 (m, 3 H, H-2, 4′, 6b′), 4.41 (dd, 1 H, *J* 3.9, 12 Hz, H-6a), 4.28 (dd, 1 H, *J* 1.5, 6.1 Hz, H-3), 4.20 (dd, 1 H, *J* 7.4, 12.0 Hz, H-6b), 4.08 (dd, 1 H, *J* 4.4, 6.0 Hz, H-4), 2.14, 1.96 (2 s, 6 H, $C(CH_3)_2$), 1.59, 1.38 (2 s, 6 H, 2 $COCH_3$). Anal. Calcd for $C_{47}H_{46}O_{17}$: C, 63.94; H, 5.25. Found: C, 63.76; H, 5.30.

3.6. 2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl-(1 → 3)-5,6-di-*O*-acetyl-D-galactopyranose (9)

Compound **8** (1.6 g, 1.81 mmol) was treated with 10:1 $CHCl_3$–CF_3COOH (30 mL) at rt for 2 h, at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete. The solution was diluted with toluene (100 mL), and the mixture was concentrated under vacuum. The residue was purified by flash chromatography (1:1 petroleum ether–EtOAc) to give **9** as a mixture of α and β isomers (1:3) (1.37 g, 90%): $[\alpha]_D$ −7.8° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.09–7.23 (m, 20 H, 4 Ph*H*), 6.11 (m, 1 H, H-5′), 5.75 (s, 0.75 H, H-1′ for β isomer), 5.68 (d, 0.25 H, *J* 5.0 Hz, H-3′ for α isomer), 5.62 (d, 0.75 H, *J* 5.0 Hz, H-3′ for β isomer), 5.55 (s, 0.25 H, H-1′ for α isomer), 5.48 (d, 0.75 H, *J* 1.1 Hz, H-2′ for β isomer), 5.43 (d, 0.25 H, *J* 1.1 Hz, H-2′ for α isomer), 5.37 (m, 0.25 H, for α isomer), 5.32 (s, 0.75 H, H-1 for β isomer), 5.35–5.30 (m, 1 H, H-5 for β isomer, H-1 for α isomer), 4.83–4.74 (m, 3 H, H-4′, 6′a, 6′b), 4.40–4.00 (m, 5 H, H-2,3,4,6a,6b), 2.13, 1.93 (2 s, 4.5 H, 2 $COCH_3$ for β isomer), 2.13, 1.97 (2 s, 1.5 H, 2 $COCH_3$ for α isomer). Anal. Calcd for $C_{44}H_{42}O_{17}$: C, 62.71; H, 5.02. Found: C, 62.82; H, 4.95.

3.7. β-D-Galactofuranosyl-(1 → 3)-D-galactopyranose (1)

Compound **9** (1.6 g, 1.90 mmol) was dissolved in an NH_3-saturated CH_3OH (50 mL). After 72 h at rt, the reaction mixture was concentrated to about 10 mL, then CH_2Cl_2 (100 mL) was added. The resultant precipitate was filtered and washed four times with CH_2Cl_2 to afford **1** (624 mg, 96%) as a mixture of α and β isomers (mainly β): $[\alpha]_D$ +28.5° (*c* 0.8, H_2O); ^1H NMR (400 MHz, D_2O): δ 5.12, 4.96 (2 H-1); ^{13}C NMR (100 MHz, D_2O): δ 109.01 (C-1 for Gal*f*), 105.98 (C-1 for β-Gal*p*), 96.0 (C-1 for α-Gal*p*). ESIMS: Anal. Calcd for $C_{12}H_{22}O_{11}$: [M] 342.3. Found: $[M + H^+]^+$ 343.2.

Acknowledgements

This work was supported by the Beijing Natural Science Foundation (6021004) and National Natural Science Foundation of China (59973026 and 29905004).

References

1. Brenna, P. J.; Nikaido, H. *Annu. Rev. Biochem.* **1995**, *64*, 29–48.
2. (a) Gerold, P.; Eckert, V.; Schwarz, R. T. *Trends Glycosci. Glycotechnol.* **1996**, *8*, 265–273;
   (b) de Lederkremer, R. M.; Colli, W. *Glycobiology* **1995**, *5*, 547–552.
3. (a) Unkefer, C. J.; Gander, J. *J. Biol. Chem.* **1990**, *265*, 685–691;
   (b) Mendonca–Previato, L.; Gorin, P. A. J.; Travassos, L. R. *Infect. Immun.* **1980**, *29*, 934–939;
   (c) Groisman, J. F.; de Lederkremer, R. M. *Eur. J. Biochem.* **1987**, *165*, 327–334.
4. (a) Williams, D. H. *Nat. Prod. Rep.* **1996**, *13*, 469–472;
   (b) Gadebusch, H. H.; Stapley, E. O.; Zimmerman, S. B. *Crit. Rev. Biotechnol.* **1992**, *12*, 225–231;
   (c) Woodin, K. A.; Morrison, S. H. *Pediatr. Rev.* **1994**, *15*, 440–461.
5. Montgomerie, J. Z. *Rev. Infect. Dis.* **1979**, *1*, 736–753.
6. Whitfield, C.; Richards, J. C.; Perry, M. B.; Clarke, B. R.; MacLean, L. L. *J. Bacteriol.* **1991**, *173*, 1420–1431.
7. (a) Jarosz, S.; Krajewski, J. W.; Zamojski, A.; Duddeck, H.; Kaiser, M. *Bull. Pol. Acad. Sci. Chem.* **1985**, *33*, 181–184;
   (b) De Jongh, D. C.; Biemann, K. *J. Am. Chem. Soc.* **1964**, *86*, 67–75.
8. Morgenlie, S. *Acta Chem. Scand.* **1973**, *27*, 3609–3611.
9. Rauter, A. P.; Ramoa-Riberio, F.; Fernanders, A. C.; Figueiredo, J. A. *Tetrahedron* **1995**, *51*, 6529–6532.
10. (a) Acree, T. E.; Shallenberger, R. S.; Lee, C. Y.; Einset, J. W. *Carbohydr. Res.* **1969**, *10*, 355–361;
    (b) Acree, T. E.; Shallenberger, R. S.; Mattick, L. R. *Carbohydr. Res.* **1968**, *6*, 498–506.
11. Ning, J.; Wang, H.; Yi, Y. *Tetrahedron Lett.* **2002**, *32*, 7349–7352.
12. Tsvetkov, Y. E.; Nikolaev, A. V. *J. Chem. Soc., Perkin Trans. 1* **2000**, 889–891.
13. De Lederkremer, R. M.; Nahmad, V. B.; Varela, O. *J. Org. Chem.* **1994**, *59*, 690–692.
14. (a) Schmidt, R. R.; Michel, J. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 731–734;
    (b) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212–215.
15. (a) Cyr, N.; Perlin, A. S. *Can. J. Chem.* **1979**, *57*, 2504–2507;

(b) Marino, C.; Varela, O.; De Lederkremer, R. M. *Carbohydr. Res.* **1989**, *190*, 65–69.
16. Dmitriev, B. A.; Nikolaev, A. V.; Shashkov, A. S.; Kochetkov, N. K. *Carbohydr. Res.* **1982**, *100*, 195–206.
17. Verduyn, R.; Douwes, M.; van der Klein, P. A. M.; Mösinger, E. M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron* **1993**, *49*, 7301–7316.
18. Ning, J.; Yi, Y.; Kong, F. *Tetrahedron Lett.* **2002**, *43*, 5545–5549.