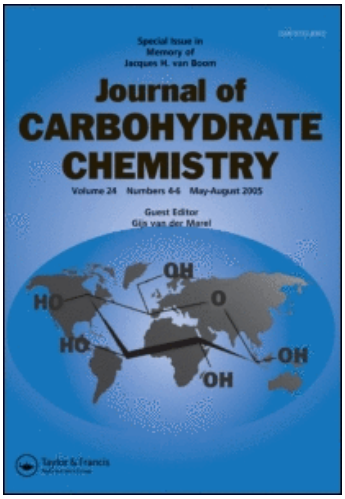

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SYNTHESIS OF LAMININ HEXASACCHARIDE ANALOGUE

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ABSTRACT

The synthesis of laminin hexasaccharide analogue di-*O*-[Gal-β-(1→4)-Glc-β]-(1→2)-(1→6)-man-α-(1→6)-man-α-Me derivative (**1**) was achieved with 1,2-anhydro-mannopyranose benzyl ether (**3**) as the key intermediate. Coupling of **3** with methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (**4**) promoted by $ZnCl_2$ gave methyl 2,3,4-tri-*O*-benzyl-6-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**2a**). Selective 6-*O*-debenzylation of **2b** with $ZnCl_2$-Ac_2O-HOAc followed by coupling with acetobromolactopyranose afforded **1**.

INTRODUCTION

Considerable effort has been devoted to the development of new methods for glycosidic coupling due to the growing importance of synthetic oligosaccharides in glycobiology.[1,2] 1,2-Anhydrosugar derivatives as glycosyl donors for building 1,2-*trans* linkages have received considerable attention recently, and they have been used in the synthesis of natural and unusual oligosaccharides.[3,4] The preparation of 1,2-anhydrosugars reported by Danishefsky's group[3] using direct epoxidation of the glycals with 3,3-dimethyl oxirane is not effective for the synthesis of 1,2-anhydrosugars such as manno- or rhamnopyranose having a *cis* arrangement of the 3-hydroxy group and the epoxide ring.

Thus, the syntheses of biologically important oligosaccharides reported by this method to date involve galacto-,[3a,3c] gluco-,[3b] xylo-,[3b] and altropyranose[3d] 1,2-anhydro derivatives, all of which have a *trans* arrangement of the 3-hydroxy group and the epoxide ring. It is known that many biologically important oligosaccarides contain manno- or rhamnopyranose residues, and that new strategies are required for their facile synthesis. Our lab have been engaged in the synthesis and glycosylation of 1,2-anhydrosugars prepared by a general method intramolecular S_N2 reaction[4] initially started by Schuerch's group.[5] We considered that 1,2-anhydromannopyranose benzyl ether synthesized by an intramolecular S_N2 reaction[4c] was an ideal glycosyl donor for the synthesis of oligosaccharides containing a 1→6 α-mannopyranose linkage, and an ideal glycosyl acceptor after its ring opening for the synthesis of 2-mono-, or 2,6-disubstituted oligosaccharides. Laminin hexasaccharide consisting of di-*O*-[Gal-β-(1→4)-GlcNAc-β]-(1→2)-(1→6)-man-α-(1→6)-man-α-Me is the core structure of laminin that can promote cell adhesion and migration and is believed to play a role in tumor cell invasion.[6] Here we present the synthesis of laminin hexasaccharide analogue di-*O*-[Gal-β-(1→4)-Glc-β]-(1→2)-(1→6)-man-α-(1→6)-man-α-Me (**1**) with 1,2-anhydromannopyranose benzyl ether as the key intermediate.

RESULTS AND DISCUSSION

The hexasaccharide **1** is composed of two lactose units and one disaccharide unit man-α-(1→6)-man-α-Me (**2**), the latter being a key unit for construction of **1**. For the synthesis of the disaccharide **2**, 1,2-anhydro-3,4,6-tri-*O*-benzyl-β-D-mannopyranose (**3**)[4c] was employed as a glycosyl donor, and is the first example of the use of 1,2-anhydromannose as the glycosyl donor in oligosaccharide synthesis. Compound **3** was prepared quantitatively as white crystals via ring closure[4c] of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride (**5**) that was prepared quantitatively by a modified route[7] via chlorination of 3,4,6-tri-*O*-benzyl-1,2-*O*-methoxyethylidene-β-D-mannopyranose (**9**)[8] with chlorotrimethylsilane. Condensation of **3** with methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (**4**)[9] in dry CH_2Cl_2 in the presence of freshly fused $ZnCl_2$ and powdered 4 Å molecular sieves gave methyl 2,3,4-tri-*O*-benzyl-6-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**2a**) in excellent yield (95%). No β-

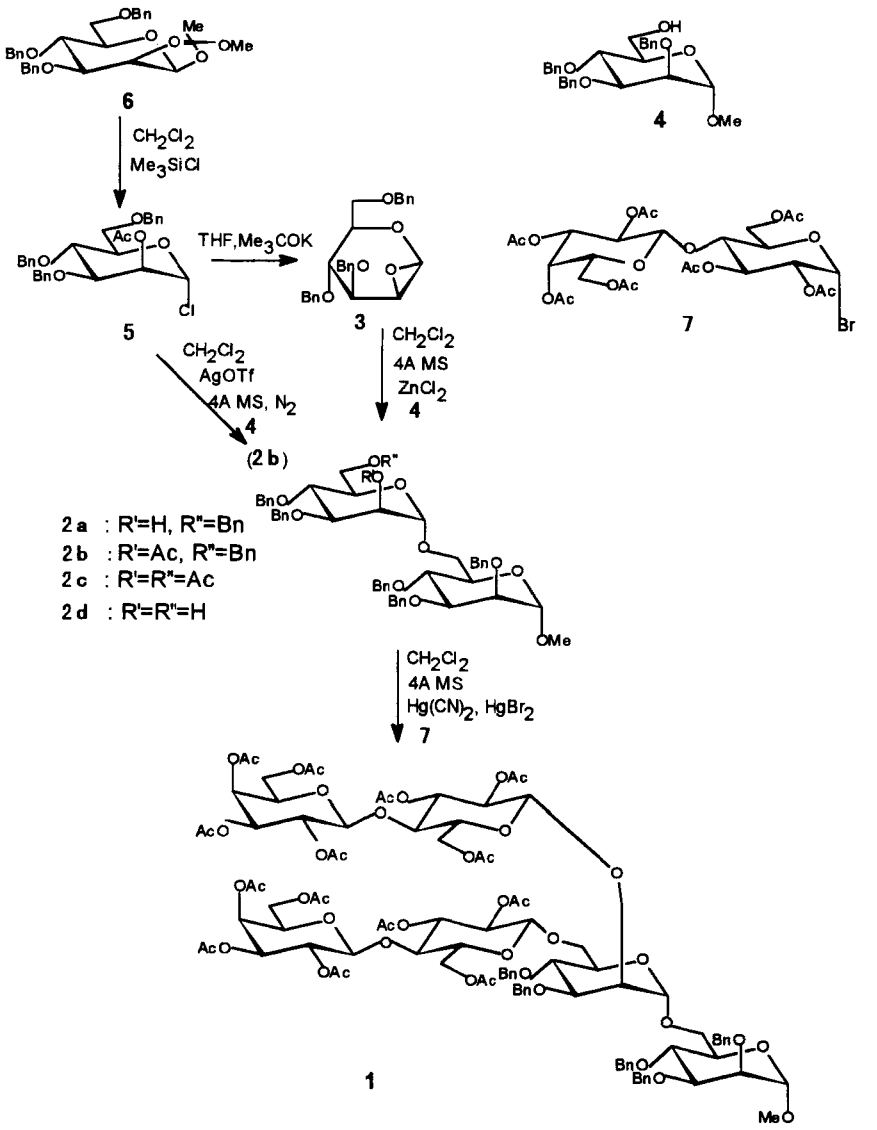
isomer was found. The best mole ratio of donor to acceptor for the coupling was 1.2 : 1. The yield and stereoselectivity did not change over a wide range of the reaction temperature (-10 °C to room temperature) and time (30 min to 18 h), and N_2 protection was not necessary. Therefore coupling with 1,2-anhydromannopyranose **3** as the glycosyl donor was carried out under easily accessible and mild coupling conditions, proceeded with high stereoselectivity and yield, and provided easy preparation and long term (several months in desiccator at 0 °C) storage of crystalline **3**.

To confirm the stereoselectivity of the coupling of **3** with **4**, a parallel study on the condensation of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride (**5**) with **4** was carried out. It is known that coupling of **5** with an appropriate glycosyl acceptor in the presence of silver triflate afforded unique α-linked oligasaccaride.[10] It was found in our research that the condensation in the presence of silver triflate gave methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**2b**) at a moderate yield. Acetylation of **2a** with acetic anhydride in pyridine offered a product identical with **2b**, and this indicated that coupling of **3** with **4** by ring opening was completely stereoselective.

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,6-di-*O*-acetyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**2c**) was obtained by selective 6-*O*-debenzylation and acetylation. Two methods were applied, i.e., treatment of **2a** or **2b** with TMSOTf in Ac_2O at -50 °C,[11] and treatment of **2a** or **2b** with $ZnCl_2$ (8 eq) in Ac_2O/HOAc (2:1 v/v) at room temperature. The latter method was recently developed in our lab.[12] Both methods can afford **2c** in satisfactory yields, but the method using $ZnCl_2$ was easier to handle. Deacetylation of **2c** with NaOMe in methanol gave **2d** (92%), and coupling of **2d** with acetobromolactose (**7**) in dichloromethane in the presence of mercuric cyanide and mercuric bromide gave the target hexasaccharide **1**[6c] in 48% yield. It is expected that the laminin hexasaccharide can be prepared by the same strategy using an appropriate lactosamine donor.

EXPERIMENTAL

General methods. Optical rotations were determined at 20 °C with a Perkin-Elmer Model 241-Mc automatic polarimeter. ^1H NMR and ^{13}C NMR spectra were



recorded with Varian XL-400 and Varian XL-200 spectrometers for solutions in $CDCl_3$ and CD_3COCD_3. Chemical shifts are given in ppm downfield from internal Me_4Si. Thin-layer chromatography (TLC) was performed on silica gel HF with detection by charring with 30% (v/v) H_2SO_4 in MeOH or in some cases by a UV detector. Analytical LC was carried out with a Gilson HPLC set consisting of two pumps (Model 306), Dynamic Mixer (Model 811c), RI Detector (Model 132), UV/VIS Detector (Model 118), stainless steel column packed with silica gel (10 × 300 mm or 4.6 × 250 mm), and an IBM computer

installed with system control software 712. Ethyl acetate - petroleum ether (bp 60-90 °C) was used as the eluent at a flow rate of 1 to 4 mL min^{-1}. Column chromatography was conducted by elution of a column (16 × 240 mm, 18 × 300 mm, 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 diminished pressure.

1,2-Anhydro-3,4,6-tri-*O*-benzyl-β-D-mannopyranose (3). To a solution of **6** (506 mg, 1 mmol) in dichloromethane (5 mL) was added chlorotrimethylsilane (0.8 mL, 6 mmol). The mixture was stirred at room temperature overnight, then concentrated and dried under vacuum to yield **5** as a light yellow syrup (510 mg, ~100%): $[\alpha]_D$ +50° (*c* 3.0, $CHCl_3$); lit[4c] $[\alpha]_D$ +56° (*c* 5.0, $CHCl_3$).

To a solution of **5** (510 mg, 1 mmol) in dry oxolane (10 mL) was added potassium *tert*-butoxide (260 mg, 2.3 mmol). The mixture was stirred at room temperature for 2 h, and concentrated to dryness under diminished pressure. The residue was repeatedly extracted with 1:2 (v/v) EtOAc - petroleum ether, and the completely colourless extracts were combined and concentrated to yield **3** as white crystals (415 mg, 96%): mp 88-90 °C; $[\alpha]_D$ +5.6° (*c* 1.1, $CHCl_3$); lit[5a] mp 89.5-90 °C; $[\alpha]_D$ +4.5° (*c* 1.0, $CHCl_3$).

Methyl 2,3,4-Tri-*O*-benzyl-6-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (2a). To a solution of **4**[9] (120 mg, 0.26 mmol) in dry dichloromethane (10 mL) was added dried 4 Å powdered molecular sieves (100 mg), the mixture was stirred for 5 min, freshly fused $ZnCl_2$ (30 mg, 0.3 mmol) was added. After stirring for another 5 min, **3** (140 mg, 0.32 mmol) was added and the mixture was kept under vigorous stirring at room temperature for about 1 h, at the end of which time TLC (1:2 v/v EtoAc - petroleum ether) showed that the starting material had completely disappeared. The mixture was filtered, the filtrate was concentrated to a syrup. Purification and separation by silica gel chromatography with 1:2 (v/v) EtOAc - petroleum ether as the eluent gave syrupy **2a** (220 mg, 95%): $[\alpha]_D$ +20.0° (*c* 3.0, $CHCl_3$); ^1H NMR δ 7.40-7.10 (m, 30H, Ph), 5.15 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1), 4.70 (d, 1H, $J_{1',2'}$ = 2.2 Hz, H-1'), 4.95-4.42 (m, 12H, $6CH_2Ph$), 4.10-3.60 (m, 12H, H-2, 2', 3, 3', 4, 4' 5, 5', 6ab, 6'ab), 3.24 (s, 3H, OCH_3), 2.90 (s, 1H, OH).

Anal. Calcd for $C_{55}H_{60}O_{11}$: C, 73.66; H, 6.70. Found: C, 73.70; H, 6.50.

Methyl 2,3,4-Tri-*O*-benzyl-6-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (2b). A mixture of **4** (209 mg, 0.45 mmol), AgOTf (250 mg, 0.96 mmol), and dried powdered 4 Å molecular sieves (1 g) in dry dichloromethane (6 mL) was stirred at 0 °C under N_2 for 30 min. A solution of **5** (253 mg, 0.50 mmol) in dry dichloromethane (4 mL) was then added dropwise to the above mixture. The mixture was stirred at room temperature for 1 h, at the end of which time TLC (1:2 v/v EtOAc - petroleum ether) indicated that the starting material had completely disappeared. After filtering, the filtrate was concentrated, the residue was chromatographed on silica gel to give **2b** as a syrup (300 mg, 71%): $[\alpha]_D$ +29.1° (*c* 5.5, $CHCl_3$); ^{1}H NMR δ 7.40-7.10 (m, 30H, Ph), 5.48 (dd, 1H, $J_{2',3'}$ = 3.1Hz, $J_{1',2'}$ = 2.0 Hz, H-2'), 4.95 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 4.69 (d, 1H, H-1'), 4.94-4.38 (m, 12H, $6CH_2Ph$), 3.55-3.95 (m, 11H, H-2, 3, 3', 4, 4', 5, 5', 6ab, 6'ab), 3.25 (s, 3H, OCH_3), 2.15 (s, 3H, $COCH_3$).

Anal. Calcd for $C_{57}H_{62}O_{12}$: C, 72.92; H, 6.61. Found: C, 72.87; H, 6.51.

Acetylation of 2a. To a solution of **2a** (200 mg, 0.2 mmol) in pyridine (1 mL) was added acetic anhydride (0.6 mL), and the solution was stirred at room temperature for 3 h, at the end of which time TLC (1:2 v/v EtOAc - petroleum ether) indicated that the reaction was complete. The reaction mixture was poured into ice water, extracted with dichloromethane, and the organic layer was dried over Na_2SO_4, concentrated, and a product identical with **2b** was obtained quantitatively.

Methyl 2,3,4-Tri-*O*-benzyl-6-*O*-(2,6-di-*O*-acetyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (2c). Method A: To a cold (-50 °C bath), stirred solution of **2a** or **2b** (90 mg, ~ 0.1 mmol) in acetic anhydride (2 mL) was added 1:3 (v/v) trimethylsilyltriflate - dichloromethane (0.15 mL), and stirring was continued for 30 min. The mixture was poured into 1:1 (v/v) dichloromethane - saturated $NaHCO_3$, and stirred for 0.5 h. The organic layer was washed with water, dried over Na_2SO_4, and concentrated. The residue was subjected to column chromatography with 1:2 EtOAc - petroleum ether as the eluent and compound **2c** (84 mg, 98%) was obtained as a syrup. Method B: To a solution of **2a** or **2b** (100 mg, ~ 0.11 mmol) in Ac_2O/HOAc (2:1 v/v, 1 mL) was added a solution of freshly fused $ZnCl_2$ (116 mg, 8 eq) in Ac_2O/HOAc (2:1 v/v, 1 mL), the mixture was stirred at room temperature for 3 h, at the end of which time TLC

(2:1 v/v petroleum ether - EtOAc) indicated that the reaction was complete. Water was added, and the mixture was extracted with dichloromethane three times, the organic layer was washed with saturated sodium carbonate, then water, and dried over Na_2SO_4, and concentrated to give a syrup. Purification of the syrup by column chromatography (2:1 v/v petroleum ether - EtOAc) yielded **2c** (90 mg, 86%) as a syrup: $[\alpha]_D$ +32.6° (*c* 3.5, $CHCl_3$); lit.[6c] $[\alpha]_D$ +34° (*c* 0.5, $CHCl_3$). ^{1}H NMR δ 7.40-7.10 (m, 30H, Ph), 5.48 (dd, 1H, $J_{2',3'}$ = 3.1 Hz, $J_{1',2'}$ = 2.0 Hz, H-2'), 4.95 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 4.70 (d, 1H, H-1'), 4.94-4.42 (m, 10H, $5CH_2Ph$), 4.40-3.70 (m, 11H, H-2, 3, 3', 4, 4', 5, 5', 6ab, 6'ab), 3.26 (s, 3H, OCH_3), 2.20,2.00 (2s, 6H, $2COCH_3$).

Methyl 2,3,4-Tri-*O*-benzyl-6-*O*-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (2d). A catalytic amount of sodium methoxide was added to a solution of **2c** (1 g, 1.1 mmol) in methanol (80 mL). The solution was allowed to stand at room temperature for 15 h, then neutralized, and concentrated to dryness. The residue was chromatographed on silica gel with EtOAc - petroleum ether (1:1 v/v) to give **2d** (830 mg, 92%) as a syrup: $[\alpha]_D$ +42.0° (*c* 4.0, $CHCl_3$); lit.[6c] $[\alpha]_D$ +47° (*c* 2, $CHCl_3$); ^{1}H NMR δ 7.40-7.10 (m, 30H, Ph), 5.15 (d, $J_{1,2}$ = 2.0 Hz, H-1), 4.70 (d, 1H, $J_{1',2'}$ = 2.2 Hz, H-1'), 4.95-4.42 (m, 10H, $5CH_2Ph$), 4.10-3.60 (m, 12H, H-2, 2', 3, 3', 4, 4' 5, 5', 6ab, 6'ab), 3.24 (s, 3H, OCH_3), 2.94 (s, 2H, 2OH).

Methyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucpyranosyl)-(1→2)-*O*-[(2,3,4,6-tetra-*O*-acetyl-β-D-galacto-pyranosyl)-(1→4)-*O*-(2,3,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→6)]-*O*-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (1). To a stirred mixture of **2d** (70 mg, 0.085 mmol), mercuric cyanide (43 mg, 0.085 mmol), mercuric bromide (7 mg, 0.017 mmol) and dried powdered 4 Å molecular sieves (500 mg) in dry dichloromethane (6 mL) was added **7** (240 mg, 0.34 mmol). The reaction mixture was stirred at room temperature overnight, diluted with dichloromethane, filtered, washed with water, dried and concentrated. The residue was chromatographed on silica gel with 1:1.5 (v/v) petroleum ether - EtOAc to give **1** (85 mg, 48%) as a syrup: $[\alpha]_D$ +33.7° (*c* 2.0, $CHCl_3$); lit.[6c] $[\alpha]_D$ +35° (*c* 1, $CHCl_3$); ^{13}C NMR δ (CD_3COCD_3) 170.38-168.84 (*C*O), 138.30-137.67, 128.36-127.43 (Ph), 102.49, 100.86, 99.41, 98.61, 96.96, 95.93 (6C-1), 54.53 (OCH_3), 20.57-20.30 ($COCH_3$).

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REFERENCES

1. R. A. Dwek, *Chem. Rev.*, **96**, 683 (1996).
2. a) K. Toshima and K. Tatsuta, *Chem. Rev.*, **93**, 1503 (1993).
   b) F. Barresi and O. Hindsgaul, *J. Carbohydr. Chem.*, **14**, 1043 (1995).
   c) R. R. Schmidt and W. Kinzy, *Adv. Carbohydr. Chem. Biochem.*, **50**, 21 (1994).
3. a) T. K. Park, I. J. Kim, S. Hu, M. T. Bilodeau, J. T. Randolph, O. Kwon, and S. J. Danishefsky, *J. Am. Chem. Soc.*, **118**, 11488 (1996).
   b) J. T. Randolph and S. J. Danishefsky, *J. Am. Chem. Soc.*, **117**, 5693 (1995).
   c) M. T. Bilodeau, T. K. Park, S. Hu, J. T. Randolph, S. J. Danishefsky, P. O. Livingston, and S. Zhang, *J. Am. Chem. Soc.*, **117**, 7840 (1995).
   d) R. G. Dushin, and S. J. Danishefsky, *J. Am. Chem. Soc.*, **114**, 655 (1992).
4. a) J. Ning and F. Kong, *J. Carbohydr. Chem.*, **16**, 311 (1997).
   b) X. Ding and F. Kong, *Carbohydr. Res.*, **286**, 161 (1996).
   c) Y. Du and F. Kong, *J. Carbohydr. Chem.*, **14**, 341 (1995).
5. a) S. J. Sondheimer, H. Yamaguchi, and C. Schuerch, *Carbohydr. Res.*, **74**, 327 (1979).
   b) H. Yamaguchi, and C. Schuerch, *Carbohydr. Res.*, **81**, 192 (1980).
6. a) S. L. Goodman, D. Newgreen, and J. B. McCarthy, S.T. Hagen, and L. T. Furcht, *J. Cell Biol*, **102**, 179 (1986).
   b) E. Rouslahti, *Cancer Metastasis Rev.*, **3**, 43 (1984).
   c) X. Zhu, P. Ding, and M. Cai, *Tetrahedron Asymmetry*, **7**, 2833 (1996).
7. T. Ogawa, K. Katano, and M. Matsui, *Carbohydr. Res.*, **64**, c3 (1978).
8. M. Mazurek and A. S. Perlin, *Can. J. Chem.*, **43**, 1918 (1965).
9. S. Soudheimer, R. Eby, and C. Schuerch, *Carbohydr. Res.*, **60**, 187 (1978).
10. T. O. Y. Nakahara, S. Shibayama, Y. Nakahara, and T. Ogawa, *Carbohydr Res.*, **280**, 67 (1996).
11. R. K. Jain and K. L. Matta, *Carbohydr. Res.*, **282**,101 (1996).
12. G. Yang, X. Ding, and F. Kong, *Tetrahedron Lett.*, 6725 (1997).