ELSEVIER

Carbohydrate Research 277 (1995) 171–178

CARBOHYDRATE RESEARCH

Note

Stereoselective preparation of alkyl glycosides of 2-acetamido-2-deoxy-α-D-glucopyranose by nonclassical halide-ion catalysis and synthesis and NMR spectroscopy of α-D-Gal *p*-(1 → 3)-α-D-Glc *p*NAc-OMe

Vince Pozsgay [a,b,*], Bruce Coxon [c]

[a] *Laboratory of Developmental and Molecular Immunity, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA*

[b] *Laboratory of Medicinal Chemistry, National Institute of Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA*

[c] *Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA*

Received 15 February 1995; accepted 4 May 1995

Keywords: Alkyl glycosides; 2-Acetamido-2-deoxy-α-D-glucopyranose; α-D-Gal *p*-(1 → 3)-α-D-Glc *p*NAc-OMe

Due to the frequent occurrence of 2-acetamido-2-deoxy-D-glucopyranose in glycoconjugates of biological interest [1], considerable research has been devoted to the development of stereoselective methods for glycosylation with this residue, as reviewed by Banoub and colleagues [2]. Unfortunately, the conditions of glycosylation reactions are not always compatible with the Lewis-basic acetamido group [3]. For example, the acetamido group at C-2 may interact with the activated anomeric center leading to oxazolines and can also be a potential glycosylation site. The azido group has been recognized as an excellent source of the acetamido group, and precursors containing this functionality were used for the synthesis of both α- and β-linked 2-acetamido-2-deoxy-glucopyranosides [4]. Glycosylations of reactive, primary alcohols with 2-azido-2-deoxy-β-D-glucopyranosyl donors lead to the corresponding β-glucopyranosides in a stereocontrolled manner in excellent yields when insoluble silver salts are used as

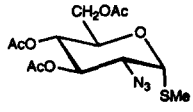
* Corresponding author. Tel: (301) 402-0036, Fax: (301) 402-9108, Email: vipo@helix.nih.gov.

Elsevier Science Ltd.
SSDI 0008-6215(95)00198-0

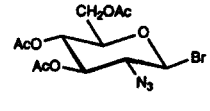
promoters. On the other hand, no stereoselective route to simple glycosides of 2-azido-2-deoxy-α-D-glucopyranose has been reported to date [2]. In connection with our interest [5] in oligosaccharides related to the O-polysaccharide (**A**) of the Gram-negative pathogen *Shigella dysenteriae* type 1 which contains, inter alia, an α-linked 2-acetamido-2-deoxy-D-glucopyranosyl residue [6], we developed a stereoselective pathway to such compounds, which we describe here.

$$[3)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow 2)\text{-}\alpha\text{-D-Gal}p\text{-}(1\rightarrow 3)\text{-}\alpha\text{-D-}GlcpNAc\text{-}(1\rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow]_n \quad \textbf{(A)}$$

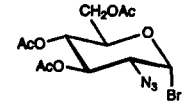
Lemieux et al. demonstrated that tetra-*O*-benzyl-β-D-glucopyranosyl bromide generated in situ from the synthetically accessible α-halide by tetraethylammonium bromide reacts with alcohols without heavy metal catalysis to yield α-glycosides in a highly stereoselective manner. The α-halide is unreactive under such conditions [7]. The in situ anomerization by tetraethylammonium halides could not be applied to 2-azido-2-deoxy-α-D-glycosyl halides [4]. We reasoned that this problem could be solved by an alternative approach to β bromides. Based on early studies by Weygand and Ziemann [8], we have shown [9] that brominolysis of a 1-thio-α-glucoside containing a nonparticipating benzyl group at O-2 affords the corresponding β-bromide in a stereocontrolled manner. We surmised that brominolysis of 2-azido-2-deoxy-1-thio-α-D-glucopyranoside **1** [10] would result in a similar stereochemical outcome.



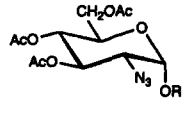
1



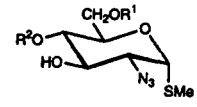
2



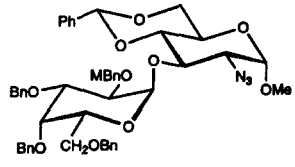
3



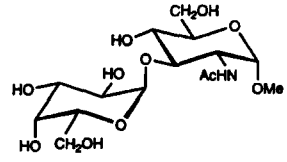
4 R=Me
5 R=All
6 R=SE



7 $R^1=R^2=H$
8 R^1,R^2=PhCH



9



10

All=allyl
SE=2-(trimethylsilyl)ethyl

Indeed, when compound **1** was reacted with bromine at 22°C, β-bromide **2** was formed rapidly, together with traces of the α-bromide **3** (^{1}H NMR). In situ treatment of the glucosyl bromides so formed with the alcohols listed in Table 1 led to a quick disappearance of the β-bromide **2**, and to formation of the α-glucosides **4–6**. Simultaneous formation of the α-bromide **3** ($\delta_{H\text{-}1} = 6.43$) was also observed. For example, in the reaction of **2** and MeOH, the glucoside **4** and the α-bromide **3** were present in a ratio of ~ 2:1 after 1 h. Since self-anomerization of the β-bromide is slow (^{1}H NMR), the quick appearance of **3** is likely to be caused by an α attack at C-1 of **2** by the bromide ion liberated in the reaction of **2** and the alcohol component. The intermediate α-bromide **3** eventually disappeared from the reaction mixture and the α-glycosides **4–6** were isolated by chromatography in 68–85% overall yields (Tables 1 and 2). Based on NMR measurements of fractions other than those of the major products it is estimated that the isomeric β-glycosides were formed in less than 5% yield. However, these products could not be isolated in pure form, and could not be positively identified. We call attention to the fact that a large excess (15–50 equivalents) of the alcohol component was necessary for the conversion of the β-bromide **2** into the α-glycosides **4–6** within acceptable reaction times, and therefore this method is limited to simple alcohol acceptors. Since an α-glucosyl bromide is unreactive towards an alcohol in the absence of a heavy metal catalyst [7], it is likely that **3** was converted to the glycosides **4–6** through the intermediacy of the β-bromide **2**. While in the classical cases of halide-ion catalysis conversion of an α-glucosyl bromide to the β anomer is brought about by exogenous bromide ion [7], regeneration of **2** from **3** must have been caused by the bromide ion already present in the reaction mixture as the hydrogen bromide salt of Hünig's base. In order to differentiate the halide ion-catalyzed glycosylation reactions brought about by endogenous bromide ion from the classical halide-ion catalysis [7], we propose the term nonclassical halide-ion catalysis for glycosylations assisted by endogenous halide ion.

Compound **4** was utilized as the key starting material for the synthesis of the methyl glycoside (**10**) of the Gal-GlcNAc fragment of the O-polysaccharide of *S. dysenteriae* type 1. Several approaches to this disaccharide have been described using various partially protected derivatives of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside as the reducing-end moiety [11]. These efforts suffered from the poor solubility of the

Table 1
Yields, specific rotations and analytical data for 2-azido-2-deoxy-α-D-glucopyranosides **4–6** [a]

Compound	Nucleophile	Reaction time (h)	Yield (%)	$[\alpha]_D$ (°)	Analytical data			
					Calcd		Found	
					C	H	C	H
4	MeOH	4	85	+169	45.22	5.55	45.08	5.57
5	AllOH [b]	24	68	+169	48.52	5.70	48.39	5.71
6	SEOH [c]	30	80	+127	47.32	6.77	47.84	6.99

[a] The synthesis of **4–6** is described in the general procedure in the Experimental.
[b] AllOH = allyl alcohol.
[c] SEOH = 2-(trimethylsilyl)ethanol.

Table 2
Selected ^{1}H and ^{13}C NMR chemical shifts [a] and coupling constants [b] for **2**, **4–7**, and **10**

Atom	Compound						
	2 [c,d]	**4** [c,d]	**5** [c,d]	**6** [c,d]	**7** [d,e]	**10** [e,f,g]	
						Gal [h]	GlcN [i]
H-1	5.31 (9.3)	4.88 (3.5)	5.03 (3.8)	4.99 (3.8)	4.92 (3.6)	5.420	4.738
H-2	3.83	3.38	3.32	3.30	3.52	3.802	4.053
H-3	5.10	5.47	5.48	5.49	3.79	3.757	3.889
H-4	5.02	5.04	5.02	5.02	3.47	3.981	3.730
H-5	3.76	4.01	4.03–3.99	4.04	3.64	3.851	3.692
H-6	4.13	4.07		4.08	3.76	3.713	3.779
H-6′	4.27	4.29	4.26	4.25	3.87	3.738	3.867
C-1		98.7	96.7	97.1	98.7	99.9	99.0
C-2		60.9	60.8	60.7	64.0	69.3	52.7
C-3		70.5	70.4	70.4	72.7	70.0	78.2
C-4		68.4	68.5	68.4	70.4	69.7	71.5
C-5		67.4	67.7	67.5	72.5	71.6	72.2
C-6		61.8	61.8	62.0	61.1	61.2	61.1

[a] Chemical shifts are quoted in ppm, using acetone (δ_H 2.225 ppm for protons and δ_C 31.00 ppm for carbons) as a secondary internal reference.
[b] Data in parentheses are three bond coupling constants in Hz.
[c] In $CDCl_3$.
[d] At 300 MHz for protons and 75.5 MHz for carbons.
[e] In D_2O.
[f] At 600 MHz for protons and 100.6 MHz for carbons.
[g] δ_{CH_3O}: 3.403; δ_{CH_3CON} = 2.052; δ_{CH_3O}: 55.9; δ_{CH_3CON} = 22.8; δ_{CH_3CON} = 174.9.
[h] H-H coupling constants (Hz): $J_{1,2}$ 3.9; $J_{2,3}$ 10.4; $J_{3,4}$ 3.2; $J_{4,5}$ 1.4; $J_{5,6}$ 7.0; $J_{5,6'}$ 5.8; $J_{6,6'}$ −11.3.
[i] H-H coupling constants (Hz): $J_{1,2}$ 3.6; $J_{2,3}$ 10.6; $J_{3,4}$ 8.3; $J_{4,5}$ 10.1; $J_{5,6}$ 5.0; $J_{5,6'}$ 2.1; $J_{6,6'}$ −12.2.

GlcNAc derivatives in ether, which was shown to be the solvent of choice for α-galactosylation [4]. No such problems were anticipated for 2-azido-2-deoxy-D-glucopyranose derivatives. Indeed, the partially protected derivative **8** [12], obtained from the triacetate **4** through routine conversions [(i) NaOMe/MeOH (→ **7**); (ii) $PhCH(OMe)_2/H^+$] had excellent solubility in ether. Methyl trifluoromethanesulfonate-mediated reaction [13] of methyl 3,4,6-tri-*O*-benzyl-2-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside [14] with the acceptor **8** in ether afforded the disaccharide derivative **9** in 84% yield. One-pot removal of the protecting groups and reduction of the azido functionality followed by *N*-acetylation afforded the target disaccharide glycoside **10** in 93% yield.

The identity and purity of the disaccharide **10** were established by NMR and mass spectroscopy. The 600-MHz ^{1}H NMR spectrum of **10** contained 10 multiplets within a region of 0.23 ppm. Due to the extensive spectral overlap, the 2D COSY spectrum was not suitable for complete assignment. A better separation of signals was seen in the 1D and 2D TOCSY spectra [15]. Fig. 1 shows the partial 1D TOCSY subspectra for the individual monosaccharide residues in **10** which were recorded under conditions described previously [14]. These spectra permitted unambiguous assignments of all protons

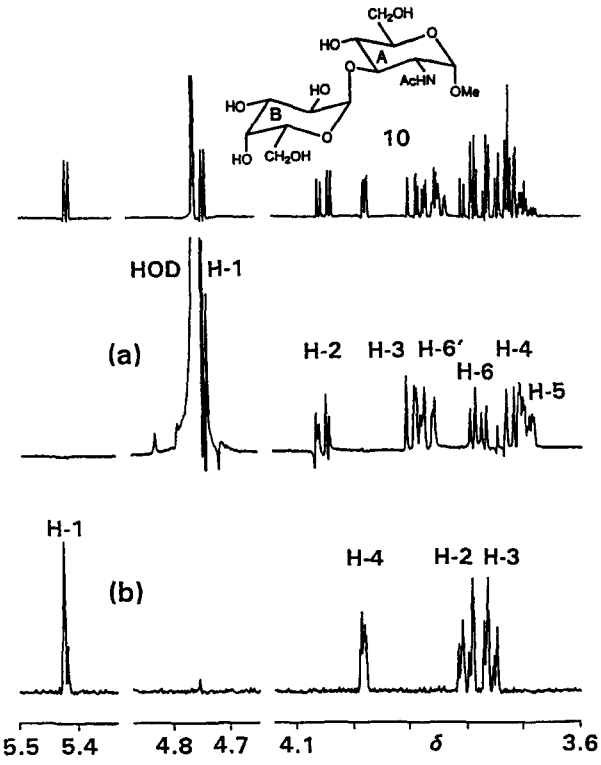


Fig. 1. ^{1}H NMR spectra of the disaccharide derivative **10** in deuterium oxide at 600 MHz. The methyl resonances are not shown. Top trace: full spectrum. Selective 1D TOCSY subspectra (a) and (b) were measured with mixing times 150 and 215 ms, respectively, and represent the 2-acetamido-2-deoxy-D-glucose (**A**) and D-galactose (**B**) residues.

for the GlcNAc residue. Most of the resonances of the Gal moiety could also be assigned. The multiplets corresponding to H-5, H-6, and H-6′ of this unit are missing because of the small value of the $^3J_{4,5}$ coupling constant which inhibits magnetization transfer from H-4 to H-5. Unambiguous assignment for these protons was made possible by the 2D TOCSY spectrum of **10**. Our assignments (Table 2) offer corrections to data published earlier [11] for several protons. The ^{13}C assignments for **10** (see Table 2) were determined by 2D HETCOR at 400 MHz. A large number of two- and three-bond CH correlations were identified by a 2D HMBC spectrum [16] of **10** measured at 360 MHz (see Experimental). In particular, this spectrum showed the structurally significant C-3_A, H-1_B and C-1_B, H-3_A correlations, in agreement with the 1,3 linkage of **10**. Several one-bond correlations were also observed due to imperfect low pass filtering, for example doublets in the F_2 dimension for C-1_A and C-1_B, $^1J_{C\text{-}1A,H\text{-}1A} = {}^1J_{C\text{-}1B,H\text{-}1B} = 171.4 \pm 0.9$ Hz.

In conclusion, an efficient route has been developed for stereocontrolled synthesis of simple glycosides of 2-azido-2-deoxy-α-D-glucopyranose. The methyl glycoside **4** thus prepared was used for the synthesis of the disaccharide α-D-Gal*p*-(1 → 3)-α-D-

Glc*p*NAc-OMe, which represents a part of the O-polysaccharide of the lipopolysaccharide of the human pathogen *S. dysenteriae* type 1.

1. Experimental

General methods.—General experimental conditions are described in [14]. Optical rotations were measured for $CHCl_3$ solutions, except where indicated otherwise, at ambient temperature. The NMR data were obtained using a Gemini 300 (Varian [1]) spectrometer, operating at 300 MHz for ^1H, and at 75.5 MHz for ^{13}C. The NMR measurements for compound **10** were performed by using Bruker AMX-360 or AMX-600 instruments for proton detection at 360 or 600 MHz, respectively, and by the use of a Bruker WM-400 spectrometer for ^{13}C detection at 100.6 MHz. The 2D HMBC spectra were acquired by proton detection at 360 MHz, using 2048 (t_2) × 512 (t_1) point data sets, zero-filled to 1024 points in the t_1 dimension, together with spectral widths of 1.801 and 9.056 kHz in the t_2 and t_1 dimensions, respectively. Internal references: Me_4Si (0.000 ppm for ^1H), acetone (2.225 ppm for ^1H and 31.00 ppm for ^{13}C for solutions in D_2O) and $CDCl_3$ (77.00 ppm for ^{13}C for solutions in $CDCl_3$). The methylene carbon resonances were identified by a DEPT-135 experiment. Subscripts A and B refer to the individual sugar residues, with A being the reducing-end unit. The ^{13}C assignments for compounds **4** and **10** are based on ^1H–^{13}C correlation maps. The fast atom bombardment mass spectra were run with a JEOL SX102 mass spectrometer, using 6 keV xenon atoms to ionize the samples, which were desorbed from a mixture of dithiothreitol and dithioerythritol. The instrument was calibrated against Ultramark 1621 (PCR Chemicals). For the low-resolution, chemical-ionization mass spectra (CIMS), ammonia was used as the ionizing gas. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

General procedure for the preparation of the glycosides **4–6**.—To a solution of methyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-α-D-glucopyranoside (**1**) [10] (1.38 mmol) in anhydrous CH_2Cl_2 (10 mL) at 22°C was added bromine (2.76 mmol). After 5 min, 1-hexene (~ 0.5 mL) was added to the solution, then most of the volatiles were removed under vacuum. The residual syrup was treated with an alcohol (3 mL) listed in Table 1, followed by ethyldiisopropylamine (2.76 mmol). After the reaction time listed in Table 1, the solution was concentrated almost to dryness, and the residue chromatographed in a mixture of EtOAc–hexane of appropriate polarity to afford the glycosides **4–6** as syrupy products.

Methyl 2-azido-2-deoxy-α-D-glucopyranoside (**7**).—A solution of **4** (160 mg) in MeOH (10 mL) was treated with a catalytic amount of NaOMe for 6 h at 25°C. The solution was treated with Dowex 50 × 2 (H^+), filtered, and concentrated to give **7** as a

[1] Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation by the National Institute of Standards and Technology, nor does it imply that the materials or equipment are necessarily the best available for the purpose.

syrup; $[\alpha]_D$ + 104° (*c* 0.5, H_2O). CIMS: *m/z* 237 $[(M + NH_4)^+]$, 191 $[(M - N_2 + H_3)^+]$. Anal. Calcd for $C_7H_{13}N_3O_5$: C, 38.36; H, 5.98; N, 19.17. Found: C, 38.43; H, 5.93; N, 19.10%.

*Methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-*D*-glucopyranoside* (**8**).—Compound **7** was treated with benzaldehyde dimethyl acetal as described for the corresponding 1-thioglycoside in ref. [10] to afford **8** as a crystalline solid (82%), which had physical properties identical to those published in [12].

Methyl O*-[3,4,6-tri-*O*-benzyl-2-*O*-(4-methoxybenzyl)-α-*D*-galactopyranosyl]-(1 → 3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-*D*-glucopyranoside* (**9**).—Methyl trifluoromethanesulfonate (100 μL) was added to a stirred mixture of methyl 3,4,6-tri-*O*-benzyl-2-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside (900 mg, 1.5 mmol), **8** (220 mg, 0.72 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (0.4 g, 1.9 mmol), 4 Å molecular sieve (0.5 g) and diethyl ether (8 mL) at 25°C. After 6 h, Et_3N (1 mL) was added. The mixture was filtered and the volatiles removed. Column chromatography of the residue, using 4:1 hexane–EtOAc as eluant, gave first methyl 3,4,6-tri-*O*-benzyl-2-*O*-(4-methoxybenzyl)-1-thio-α-D-galactopyranoside [14] (160 mg, 18%). Further elution afforded amorphous **9** (425 mg, 84%); $[\alpha]_D$ +88° (*c* 0.6); NMR ($CDCl_3$): ^1H, δ 7.40–6.59 (aromatic), 5.476 (s, 1 H, *H*CPh), 3.733 [s, 3 H, CH_3O (aromatic)], 3.450 [s, 3 H, CH_3O (aglycon)], and 3.411 (dd, 1 H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 10.2 Hz, H-2_A); ^{13}C, δ 138.8–126.1 and 113.4 (aromatic), 101.9 (*C*HPh), 99.6 ($^1J_{C\text{-}1,H\text{-}1}$ 172 Hz, C-1_B), 97.3 ($^1J_{C\text{-}1,H\text{-}1}$ 171 Hz, C-1_A), 83.1 (C-3_B), 78.4 (C-4_A), 74.9, 73.4, 73.0, and 71.7 [*C*H_2 (Bn and MBn)], 74.9, 71.3, and 69.5 (C-2_B, 3_A, 4_B, 5_A, 5_B), 68.5 and 69.0 (C-6_A, 6_B), 62.2 (C-2_A), 55.3 and 55.4 (2 *C*H_3O). FABMS: *m/z* 858 $[(M + 1 - H_2)^+]$, 832 $[(M + 1 - N_2)^+]$. Anal. Calcd for $C_{49}H_{53}N_3O_{11}$: C, 68.43; H, 6.21; N, 4.89. Found: C, 68.39; H, 6.19; N, 4.81%. Subsequent elution afforded unreacted **9** (60 mg).

Methyl O*-α-*D*-galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-α-*D*-glucopyranoside* (**10**).—A mixture of **9** (350 mg), 10% Pd—C (200 mg), EtOH (10 mL) and AcOH (0.5 mL) was stirred under hydrogen for 24 h under atmospheric pressure, at 25°C. The catalyst was removed by filtration and the solution was cooled to 0°C. Acetic anhydride (200 μL) was added. After 10 min the solution was concentrated to give **10** (150 mg, 93%). This preparation was purified by gel filtration through a column of Biogel P-2, using 0.02 M pyridinium acetate as eluant. Freeze-drying of the fractions containing carbohydrate as indicated by the phenol–H_2SO_4 assay [17] afforded **10** as an amorphous solid; $[\alpha]_D$ + 150° (*c* 0.4, H_2O), lit. [11] $[\alpha]_D$ + 161° (*c* 0.8, H_2O). For numerical NMR data, see Table 2. 2D HMBC two- and three-bond CH correlations: CH_3C, C=O; C=O, H-2_A; *C*H_3O, H-1_A; CH_3O, C-1_A; C-1_A, H-2_A; C-2_A, H-3_A; C-3_A, H-1_A; C-3_A, H-2_A; C-3_A, H-1_B; C-3_A, H-4_A; C-3_A, H-5_A; C-4_A, H-3_A; C-5_A, H-1_A; C-5_A, H-3_A; C-5_A, H-4_A; C-5_A H-6_A; C-1_B, H-3_A; C-1_B, H-5_B; C-2_B, H-1_B; C-2_B, H-3_B; C-2_B, H-4_B; C-3_B, H-1_B; C-3_B, H-2_B; C-3_B, H-4_B; C-4_B, H-3_B; C-4_B, H-5_B; C-4_B, H-6_B; C-5_B, H-1_B; C-5_B, H-6_B; C-6_B, H-5_B. FABMS: *m/z* 795 $[(2M + 1)^+]$, 398 $[(M + 1)^+]$.

Acknowledgements

We thank Dr Lewis Pannell and Mr Noel Whittaker for the mass spectra.

References

[1] H.J. Allen and E.C. Kisalius (Eds.), *Glycoconjugates, Composition, Structure, and Function*, Marcel Dekker, 1992.
[2] J. Banoub, P. Boullanger, and D. Lafont, *Chem. Rev.*, 92 (1992) 1167–1195.
[3] S. Knapp, C. Jaramillo, and B. Freeman, *J. Org. Chem.*, 59 (1994) 4800–4804.
[4] H. Paulsen, *Angew. Chem., Int. Ed. Engl.*, 21 (1982) 155–173.
[5] V. Pozsgay, *J. Am. Chem. Soc.*, 117 (1995) 6673–6681, and references therein.
[6] (a) B.A. Dmitriev, Yu.A. Knirel, N.K. Kochetkov, and I.L. Hofman, *Eur. J. Biochem.*, 66 (1976) 559–566; (b) S. Sturm, B. Jann, K. Jann, P. Fortnagel, and K.N. Timmis, *Microbial Pathogen.*, 1 (1986) 307–324.
[7] R.U. Lemieux, K.B. Hendricks, R.V. Stick, and K. James, *J. Am. Chem. Soc.*, 97 (1975) 4056–4062.
[8] F. Weygand and H. Ziemann, *Justus Liebigs Ann. Chem.*, 657 (1962) 179–198.
[9] V. Pozsgay and J.B. Robbins, *Carbohydr. Res.*, 277 (1995) 51–66.
[10] V. Pozsgay, C.P.J. Glaudemans, J.B. Robbins, and R. Schneerson, *Tetrahedron*, 48 (1992) 10249–10264.
[11] P. Kovac and K.J. Edgar, *J. Org. Chem.*, 57 (1992) 2455–2467.
[12] V. Pozsgay and B. Coxon, *Carbohydr. Res.*, 257 (1994) 189–215.
[13] H. Lönn, *Carbohydr. Res.*, 139 (1985) 105–113.
[14] V. Pozsgay, B. Coxon, and H. Yeh, *Bioorg. Med. Chem.*, 1 (1993) 237–257.
[15] A. Bax and D.G. Davis, *J. Magn. Reson.*, 65 (1985) 355–360.
[16] A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, 108 (1986) 2093–2094.
[17] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.