ELSEVIER

Carbohydrate Research 310 (1998) 145–149

CARBOHYDRATE RESEARCH

Note

Improved synthesis of an aldobiouronic acid related to hardwood xylans, and preparation of a derivative thereof suitable for linking to proteins

Ján Hirsch [a], Miroslav Kóš [a], Pavol Kováč [b,*]

[a] *Institute of Chemistry, Slovak Academy of Sciences, SK-842 38 Bratislava, Slovak Republic*
[b] *NIDDK, National Institutes of Health, Bethesda, Maryland 20892-0815, USA*

Received 20 April 1998; accepted 12 June 1998

Abstract

Treatment of 1,3,4-tri-*O*-acetyl-α-D-xylopyranose with methyl 2,3-di-*O*-benzyl-1-chloro-1-deoxy-4-*O*-methyl-α, β-D-glucopyranuronate in the presence of silver trifluoromethanesulfonate was highly stereoselective to give the α-linked aldobiouronic acid derivative (**4**) in 86% yield, after hydrogenolysis of the crude product of the coupling and chromatography. Compound **4** was acetylated and the fully protected substance was converted to the corresponding glycosyl chloride. Reaction of the latter with *p*-nitrophenol under phase-transfer catalysis afforded, after deacetylation, *p*-nitrophenyl 2-*O*-(methyl 4-*O*-methyl-α-D-glucopyranosyluronate)-β-D-xylopyranoside.

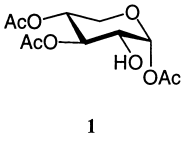
Keywords: Aldobiouronic acid; Stereoselective α-glycosylation; Xylopyranosides; Disaccharides; Neoglycoconjugate

Polysaccharides in plant tissues can be localized, inter alia, using specific antibodies. Such histochemical probes have been prepared using as immunogens glycoconjugates carrying determinant epitopes [1]. (4-*O*-Methylglucurono)xylans are important constituents of cell-wall polysaccharides of woods and other plants. These biopolymers are composed mainly of (1→4)-β-linked D-xylopyranoses, some of which are randomly branched at position O-2 with 4-*O*-methyl-α-D-glucopyranosyluronic acid. Aldobiouronic acids that reflect these structural features have been isolated from products of partial hydrolysis of hemicelluloses from hardwoods [2,3], and the lowest oligosaccharide of this class, 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose (**3**) [4], as well as its methyl β-glycoside [5,6], have been synthesized. The preparation of **3** [4] was based on the condensation of methyl 2,3-di-*O*-benzyl-1-chloro-1-deoxy-4-*O*-methyl-α, β-D-glucopyranuronate (**2**) [6] and benzyl 3,4-di-*O*-benzyl-β-D-xylopyranoside. The preparation of this nucleophile [7] requires several steps, making the synthesis of **3** tedious.

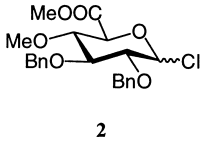
Takahashi and Sumyia [8] have prepared an immunogen from **3** isolated from wood xylan.

* Corresponding author. Fax: +1-301-402-0589; e-mail: kpn@helix.nih.gov

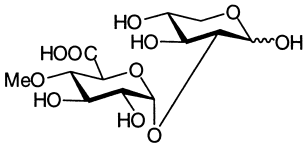
0008-6215/98/$—see front matter
PII S0008-6215(98)00161-X



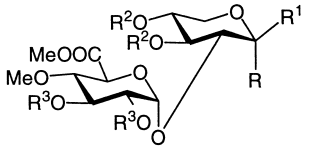
1



2



3



	R	R^1	R^2	R^3
4	OAc	H	Ac	H
5	OAc	H	Ac	Ac
6	H,OH		H	H
7	Cl	H	Ac	Ac
8	H	$OC_6H_4NO_2$	Ac	Ac
9	H	$OC_6H_4NO_2$	H	H

Projects ongoing in our laboratories, involving among other things raising antibodies to (4-*O*-methylglucurono)xylans, require a large amount of **3**. Compound **3** has now been made more easily available by synthesis, by using 1,3,4-tri-*O*-acetyl-α-D-xylopyranose (**1**) [9] as a nucleophile in the condensation reaction with **2**. Compound **1** can be obtained from D-xylose by a one-pot operation, curtailing the synthesis of **3** by six steps compared with that described previously [4]. Accordingly, compounds **1** and **2** were coupled under conditions of silver trifluoromethanesulfonate (triflate)-mediated glycosylation, and the crude product was subjected to hydrogenolysis. The crystalline, α-linked product **4**, was obtained in 86% yield, showing glycosylation to be highly stereoselective. It compares well with the high stereoselectivity observed during preparation of the corresponding methyl β-glycoside (85.8% yield of the α-linked disaccharide [5,6]) where, as in the present case, the nucleophile contained acetyl protecting groups. In contrast, in the original synthesis of **3** [4], where the nucleophile bore benzyl protecting groups, the glycosylation reaction was much less stereoselective yielding products in a ratio of $\alpha:\beta\sim3:1$. These results are in an excellent agreement with the earlier observation [10] that the use of less reactive nucleophiles in glycosylations, such as those containing acyl as opposed to alkyl substituents, favors 1,2-cis-stereoselectivity of glycosylation. After deprotection of **4**, product **3** obtained showed physical constants identical with those observed for the material isolated from wood xylans [3].

Preparation of an immunogen from **3** required conversion of substance **4** into a derivative suitable for linking to proteins. Therefore, compound **4** was acetylated to give **5**, and the fully protected disaccharide was converted to the corresponding α-glycosyl chloride **7** by treatment with dichloromethyl methyl ether (DCMME)/$ZnCl_2$ reagent [11]. Treatment of **7** with *p*-nitrophenol under acid catalysis [12] did not produce the desired *p*-nitrophenyl glycoside **8** in a satisfactory yield, but phase-transfer catalysis [13] was successful. Finally, Zemplén O-deacetylation of **8** afforded the desired aldobiouronic acid derivative **9**.

1. Experimental

General methods.—Melting points were determined on a Kofler hot-stage. Optical rotations (at 20 °C) were measured with a Perkin–Elmer Model 241 automatic polarimeter (10 cm cell). Microanalyses were performed with a Fisons EA 1108 analyzer. ^1H (300.13 MHz) and ^{13}C (75.46 MHz) NMR spectra (internal standard Me_4Si) were recorded with a Bruker AM 300 and Bruker AVANCE DPX 300 spectrometers (equipped with gradient-enhanced spectroscopy kit GRASP for generation of the Z gradient up to 50 Gauss/cm). For 2D experiments, HMQC and COSY-45 techniques were used. When reporting assignments of NMR signals, data for the methyl glucopyranuronate residue are identified by a prime and those for the aglycon by a double prime. The EI and CI (using pyridine as a reactive agent; only one peak, characteristic of the molecular weight of compound, is registered by this method [14]) mass spectra (70 eV) were obtained with a Finnigan MAT SSQ 710 spectrometer using direct sample introduction or GC–MS techniques. All reactions were monitored by TLC on silica gel G. Visualization was affected by spraying the plates with 5% (v/v) solution of H_2SO_4 in ethanol followed by heating at ca. 200 °C. Preparative chromatography was performed on dry-packed Silica Gel 60 (E. Merck) which, prior to packing, had been equilibrated with 40% of the mobile phase.

1,3,4-Tri-O-*acetyl-2*-O-*(methyl 4*-O-*methyl*-α-D-*glucopyranosyluronate)*-α-D-*xylopyranose* (**4**).—A mixture of 1,3,4-tri-*O*-acetyl-α-D-xylopyranose (**1**) [9] (1.0 g, 3.62 mmol), silver trifluoromethanesulfonate (1.2 g, 4.68 mmol) and 2,4,6-trimethylpyridine (0.45 mL, 3.3 mmol) in dry diethyl ether (100 mL) was vigorously stirred for 30 min. A solution of methyl 2,3-di-*O*-benzyl-1-chloro-1-deoxy-4-*O*-methyl-D-glucopyranuronate (**2**) [6] (2.0 g, 4.74 mmol) in diethyl ether (15 mL) was added dropwise at −15 °C, and the reaction mixture was stirred for another 1 h at room temperature. TLC (15:1 toluene–acetone) then showed that both **1** (R_f 0.10) and the glycosyl chloride (R_f 0.60) had been consumed, and that 1,3,4-tri-*O*-acetyl-2-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-methyl-α-D-glucopyranosyluronate)-α-D-xylopyranose (R_f 0.30) was formed as the main product. Small amount of (1→2)-β-linked disaccharide (R_f 0.25), a product of hydrolysis of the chloride (R_f 0.20), and a non-reducing (1→1) disaccharide (R_f 0.35) were also present. After filtration, the filtrate was washed successively with aqueous sodium bicarbonate, sodium thiosulfate and water, and concentrated. A solution of the residue in 1:5 acetone–methanol (180 mL) was hydrogenated at room temperature over 5% palladium-on-charcoal catalyst (0.5 g) for 4 h. Conventional processing, column chromatography (R_f 0.50, 1:1 toluene–acetone), and crystallization afforded **4** (1.5 g, 86%). Recrystallization gave material, mp 175–177 °C (from diethyl ether), $[\alpha]_D$ +139° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 6.33 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 5.39 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.7 Hz, H-3), 4.98 (ddd, 1 H, $J_{4,5a}$ 5.9 Hz, $J_{4,5b}$ 10.8 Hz, H-4), 4.97 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 4.05 (d, 1 H, $J_{4',5'}$ 10.0 Hz, H-5′), 3.93 (dd, 1 H, H-2), 3.89 (dd, 1 H, $J_{5a,5b}$ 10.8 Hz, H-5a), 3.83 (s, 3 H, $COOCH_3$), 3.71 (t, 1 H, $J_{4,5b}$ 10.8 Hz, H-5b), 3.60 (t, 1 H, $J_{2',3'} = J_{3',4'}$ 8.6 Hz, H-3′), 3.53 (dd, 1 H, H-2′), 3.48 (s, 3 H, OCH_3), 3.32 (dd, 1 H, H-4′) 2.19, 2.10, and 2.04 (3 s, each 3 H, 3 Ac); ^{13}C NMR ($CDCl_3$): 170.4, 170.1, 169.7 (2 C) (4 CO ester), 98.4 (C-1′), 89.4 (C-1), 80.8 (C-4′), 75.1 (C-2), 73.6 (C-3′), 71.7 (C-2′), 70.6 (C-5′), 70.3 (C-3), 68.5 (C-4), 60.8 (C-5), 60.5 (OCH_3), 52.6 ($COOCH_3$), 21.0, 20.8, and 20.6 (3 $COCH_3$); EIMS *m*/*z* (I_r%) 259 (10), 245 (62), 205 (51), 187 (83), 157 (30), 127 (27), 97 (58), 87 (100), 85 (36), 43 (88); CIMS *m*/*z* 560 (100%, $[M + C_5H_5NH]^+$). Anal. Calcd for $C_{19}H_{28}O_{14}$: C, 47.50; H, 5.87. Found: C, 47.50; H, 5.62.

1,3,4-Tri-O-*acetyl-2*-O-*(methyl 2,3-di*-O-*acetyl-4*-O-*methyl*-α-D-*glucopyranosyluronate)*-α-D-*xylopyranose* (**5**).—Compound **4** (1.3 g) was treated with a mixture 4:1 acetic anhydride–pyridine (25 mL) for 6 h at room temperature. Conventional processing and crystallization afforded **5** (1.4 g, 92%); mp 84–86 °C (from ethanol); $[\alpha]_D$ +118° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 6.20 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.46 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.8 Hz, H-3), 5.31 (dd, 1 H, $J_{2',3'}$ 10.3 Hz, $J_{3',4'}$ 9.3 Hz, H-3′), 5.09 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 4.97 (ddd, 1 H, $J_{4,5a}$ 6.0 Hz, $J_{4,5b}$ 10.9 Hz, H-4), 4.85 (dd, 1 H, H-2′), 4.26 (d, 1 H, $J_{4',5'}$ 9.9 Hz, H-5′), 3.89 (dd, 1 H, H-2), 3.84 (s, 3 H, $COOCH_3$), 3.83 (dd, 1 H, $J_{5a,5b}$ 10.9 Hz, H-5a), 3.70 (t, 1 H, H-5b), 3.57 (dd, 1 H, H-4′), 3.38 (s, 3 H, OCH_3), 2.21, 2.11, 2.06, 2.05, and 2.02 (5 s, each 3 H, 5 Ac); ^{13}C NMR ($CDCl_3$): δ 169.9 (2 C), 169.8, 169 .4, 169.0 (2 C) (6 CO ester), 95.9 (C-1′), 88.5 (C-1), 78.9 (C-4′), 74.3 (C-2), 70.7 (C-3′), 70.1 (C-5′), 70.0 (C-2′), 69.9 (C-3), 68.8 (C-4), 60.4 (C-5), 60.0 (OCH_3), 52.6 ($COOCH_3$), 20.7 (2 C), 20.6 (2 C), and 20.4 (5 $COCH_3$); EIMS *m*/*z* (I_r/%) 373 (10), 289 (78), 229 (63), 187 (100), 140 (17), 127 (17), 97 (16), 85 (15), 43 (66); CIMS *m*/*z* 644 (100%, $[M + C_5H_5NH]^+$). Anal. Calcd for $C_{23}H_{32}O_6$: C, 48.93; H, 5.71. Found: C, 49.06; H, 5.67.

2-O-*(Methyl 4*-O-*methyl*-α-D-*glucopyranosyluronate)*-D-*xylopyranose* (**6**).—A solution of 4 (0.5 g) in dry methanol (15 mL) was treated with a catalytic amount of methanolic NaOMe. The mixture was kept for 2 h at room temperature, neutralized with Dowex-50 W (H^+) resin, filtered, and concentrated. The residue was crystallized from ethanol to give **6** (0.32 g, 87%); mp 174–175 °C; $[\alpha]_D$ +132° (extrapolated)→+108° (90 min, equil, *c* 1, H_2O); lit. [4] mp 174–176 °C; $[\alpha]_D$ +133°→106° (90 min, equil).

p-*Nitrophenyl 3,4-di*-O-*acetyl-2*-O-*(methyl 2,3-di*-O-*acetyl-4*-O-*methyl*-α-D-*glucopyranosyluronate)*-β-D-*xylopyranoside* (**8**).—Disaccharide **5** (1.0 g, 1.78 mmol) in dichloromethyl methyl ether (5 mL) containing a catalytic amount of freshly fused zinc chloride was heated at 50 °C for 1 h. TLC (7:1 chloroform–ethyl acetate) then showed that all starting material was converted to a major product **7** (R_f 0.65). The reaction mixture was concentrated at reduced pressure and the solution of the residue in chloroform was washed with cold, satd aq solution of sodium bicarbonate. Evaporation of the solvent under diminished pressure gave **7** (0.95 g, ~100%) as a colourless oil, sufficiently pure for the next step. ^{1}H NMR ($CDCl_3$): δ 6.00 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 5.42 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.7 Hz, H-3), 5.30 (t, 1 H, $J_{2',3'} = J_{3',4'}$ 10.2 Hz, H-3′), 5.19 (d, 1

H, $J_{1',2'}$ 3.7 Hz, H-1′), 4.90 (dt, 1 H, $J_{4,5a}$ 6.7 Hz, $J_{4,5b}$ 9.7 Hz, H-4), 4.63 (dd, 1 H, H-2′), 4.16 (d, 1 H, $J_{4',5'}$ 10.0 Hz, H-5′), 3.91 (dd, 1 H, H-2), 3.86 (dd, 1 H, $J_{5a,5b}$ 9.7 Hz, H-5a), 3.77 (s, 3 H, $COOCH_3$), 3.50 (t, 1 H, H-5b), 3.34 (dd, 1 H, H-4′), 3.31 (s, 3 H, OCH_3), 2.05, 2.02, 2.00, and 1.98 (4 s, each 3 H, 4 Ac); ^{13}C NMR ($CDCl_3$): 170.5, 169.9, 169.6, 169.2, 168.9 (5 CO ester), 93.8 (C-1′), 90.6 (C-1), 78.8 (C-4′), 73.8 (C-2), 70.6 (C-3′), 70.4 (C-5′), 69.9 (C-2′), 69.5 (C-3), 68.3 (C-4), 60.8 (C-5), 59.9 (OCH_3), 52.6 ($COOCH_3$), 20.7, 20.6, and 20.5 (2 C) (4 $COCH_3$); EIMS m/z (I_r/%) 389 (12), 349 (51), 307 (22), 289 (41), 229 (38), 117 (36), 87 (26), 43 (100).

To a solution of the foregoing chloride **7** (0.95 g, 1.76 mmol), tetrabutylammonium hydrogen sulfate (0.6 g, 1.76 mmol), and *p*-nitrophenol (0.5 g, 3.6 mmol) in dichloromethane (10 mL) was added 1 M sodium hydroxide (10 mL), and the mixture was vigorously stirred at room temperature for 20 h. TLC (7:1 chloroform–ethyl acetate) then showed that chloride **7** was consumed and that a large amount of **8** (R_f 0.6) was formed. Ethyl acetate (50 mL) was added, the organic phase was separated, and washed successively with 1 M NaOH (three times), water (twice), and brine. After drying (Na_2SO_4), the solvents were evaporated at reduced pressure, and the crude product was purified by column chromatography (silica gel, 12:1 chloroform–ethyl acetate) to give **8** (0.8 g, 71%); mp 183–185 °C (from ethanol); $[\alpha]_D$ +38° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.06–8.33 (m, 4 H, aromatic), 5.54 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1′), 5.35 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, $J_{3',4'}$ 9.4 Hz, H-3′), 5.34 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.2 Hz, H-3), 5.22 (d, 1 H, $J_{1,2}$ 6.9 Hz, H-1), 5.02 (dt, 1 H, $J_{3,4} = J_{4,5b}$ 9.2 Hz, $J_{4,5a}$ 5.3 Hz, H-4), 4.84 (dd, 1 H, H-2′), 4.25 (d, 1 H, $J_{4',5'}$ 10.0 Hz, H-5′), 4.15 (dd, 1 H, $J_{5a,5b}$ 11.8 Hz, H-5a), 4.00 (dd, 1 H, H-2), 3.85 (s, 3 H, $COOCH_3$), 3.59 (dd, 1 H, H-4′), 3.57 (dd, 1 H, H-5b), 3.38 (s, 3 H, OCH_3), 2.16, 2.08, 2.05, and 1.52 (4 s, each 3 H, 4 Ac); ^{13}C NMR ($CDCl_3$): δ 169.9 (2 C), 169.7, 169.4, 169.0 (5 CO ester), 160.6 (C-1″), 143.1 (C-4″), 125.7 (2 C) (C-3″, C-5″), 116.5 (2 C) (C-2″, C-6″), 100.0 (C-1), 96.2 (C-1′), 78.9 (C-4′), 75.5 (C-2), 71.6 (C-3), 70.5 (C-3′), 70.3 (2 C) (C-2′, C-5′), 69.1 (C-4), 62.7 (C-5), 59.9 (OCH_3), 52.7 ($COOCH_3$), 20.7, 20.6 (2 C), and 20.0 (4 $COCH_3$); CIMS m/z 723 (100%, $[M + C_5H_5NH]^+$). Anal. Calcd for $C_{27}H_{33}NO_{17}$: C, 50.39; H, 5.17; N, 2.18. Found: C, 50.48; H, 5.29; N, 2.16.

p-*Nitrophenyl 2*-O-*(methyl 4*-O-*methyl*-α-D-*glucopyranosyluronate)*-β-D-*xylopyranoside* (**9**).—De-*O*-acetylation of **8** (0.5 g), as described for the preparation of **6**, gave pure **9** (0.33 g, 89%); mp 139–141 °C (from ethanol); $[\alpha]_D$ +32° (*c* 0.5, $CHCl_3$). ^{1}H NMR (CD_3OD): δ 8.27 (d, 2 H, $J_{2'',3''} = J_{5'',6''}$ 9.1 Hz, H-3″ and H-5″), 7.28 (d, 2 H, H-2″ and H-6″), 5.42 (d, 1 H, $J_{1',2'}$ 3.6 Hz, H-1′), 5.31 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 4.80 (d, 1 H, $J_{4',5'}$ 10.0 Hz, H-5′), 4.00 (dd, 1 H, $J_{4,5a}$ 5.0 Hz, $J_{5a,5b}$ 11.3 Hz, H-5a), 3.84 (s, 3 H, COOCH3), 3.83 (t, 1 H, $J_{2',3'} = J_{3',4'}$ 9.4 Hz, H-3′), 3.70 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.3 Hz, H-3), 3.67 (dd, 1 H, H-2), 3.62 (dd, 1 H, H-2′), 3.54 (s, 3 H, OCH_3), 3.52 (dt, 1 H, $J_{4,5b}$ 9.3 Hz, H-4), 3.49 (dd, 1 H, H-5b), 3.32 (dd, 1 H, H-4′); ^{13}C NMR (CD_3OD): δ 172.7 (CO ester), 163.4 (C-1″), 144.1 (C-4″), 126.5 (2 C) (C-3″, C-5″), 118.1 (2 C) (C-2″, C-6″), 102.3 (C-1), 100.1 (C-1′), 83.3 (C-4′), 79.3 (C-3), 76.0 (C-2′), 74.1 (C-3′), 73.2 (C-4), 71.4 (C-2), 71.2 (C-5′), 67.0 (C-5), 60.6 (OCH_3), 52.8 ($COOCH_3$). Anal. Calcd for $C_{19}H_{25}NO_{13}$: C, 48.00; H, 5.30; N, 2.95. Found: C, 47.78; H, 5.42; N, 2.82.

Acknowledgements

The authors are grateful to C. P. J. Glaudemans (NIDDK) for his keen interest in this work and helpful suggestions. We thank also K. Paule, G. Košický, A. Karovičová, Dr. V. Pätoprstý, and E. Morháčová (Institute of Chemistry) for microanalyses, optical rotations, NMR and mass spectral measurements, and technical assistance, respectively. Financial support of this work by the Scientific Grant Agency (VEGA, Slovak Academy of Sciences and Ministry of Education, Bratislava, Grant No. 2/4144/97) and National Science Foundation (Grant No. NSF-INT 9507510) is gratefully appreciated.

References

[1] Y. Sone, A. Misaki, and S. Shibata, *Carbohydr. Res.*, 191 (1989) 79–89.
[2] T.E. Timell, *Adv. Carbohydr. Chem.*, 19 (1964) 247–302.
[3] T.E. Timell, *Can. J. Chem.*, 37 (1959) 827–829.
[4] P. Kováč, E. Petráková, and P. Kočiš, *Carbohydr. Res.*, 93 (1981) 144–147.
[5] P. Kováč and R. Palovčík, *Carbohydr. Res.*, 54 (1977) C11–C13.
[6] P. Kováč and R. Palovčík, *Chem. Zvesti*, 32 (1978) 501–513.

[7] P. Kováč and E. Petráková, *Chem. Zvesti*, 34 (1980) 537–542.
[8] N. Takahashi and K. Sumyia, *Wood Res.*, 77 (1990) 8–17.
[9] B. Helferich and W. Ost, *Chem. Ber.*, 95 (1962) 2616–2622.
[10] H. Paulsen, *Angew. Chem., Int. Ed. Engl.* 21 (1982) 155–173.
[11] P. Kováč, Synthesis of glucosyl halides for oligosaccharide synthesis using dihalogluomethyl methyl ethers, in *Modern Methods in Carbohydrate Synthesis*, S.H. Khan, and R.A. O'Neill (Eds.) Harwood Academic, Amsterdam, 1996, pp 55–81.
[12] E. Zissis and C.P.J. Glaudemans, *Carbohydr. Res.*, 50 (1976) 292–295.
[13] R. Roy and F.D. Tropper, *Can. J. Chem.*, 69 (1991) 817–821.
[14] V. Pätoprstý, V. Kováčik, and Š. Karácsonyi, *Rapid Commun. Mass Spectrom.*, 9 (1995) 840–844.