Available online at www.sciencedirect.com

SCIENCE DIRECT®

Carbohydrate Polymers 63 (2006) 136–140

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Short communication

New types of α-amylase enzyme-inhibitory polysaccharides from D-glucal

Sándor Kéki [a], Gyula Batta [b], Ilona Bereczki [c], Zsolt Fejes [c], Lajos Nagy [a], Ágnes Zajácz [d],
Lili Kandra [d], Imre Kiricsi [e], György Deák [a], Miklós Zsuga [a,*], Pál Herczegh [b,c,*]

[a] Department of Applied Chemistry, University of Debrecen, H-4010 Debrecen
[b] Research Group for Antibiotics of the Hungarian Academy of Sciences, University of Debrecen, Hungary
[c] Department of Pharmaceutical Chemistry, University of Debrecen, H-4010 Debrecen
[d] Department of Biochemistry, University of Debrecen, H-4010 Debrecen, Hungary
[e] Department of Applied and Environmental Chemistry University of Szeged, Hungary

Received 27 June 2005; accepted 12 July 2005
Available online 27 October 2005

Dedicated to Professor András Lipták on the occasion of his 70th birthday

Abstract

We describe the synthesis of new types of α-amylase enzyme-inhibitory polysaccharides obtained by polycondensation of 3,6-Di-O-acetyl-D-glucal followed by deacetylation. The structure of the resulting new polysaccharides containing unique 2,3-unsaturated hexopyranose repeating units were unambiguously determined by NMR and MALDI-TOF MS methods. The deacetylated polysaccharides proved to be a semicompetitive inhibitor of the human salivary amylase enzyme.

Keywords: Ferrier rearrangement; Unsaturated glycals; Polycondensation; NMR; MALDI-TOF MS

One of the most challenging topics both in the field of polymer and carbohydrate chemistry is to synthesize biologically active polysaccharides with well-controlled molecular weight and stereoselectivity. For the synthesis of polysaccharides until now the ring-opening polymerisation of anhydro sugars has been reported (Good & Schuerch, 1985; Ichikawa, Kobayashi, Sumitomo, & Schuerch, 1988; Kobayashi, Ishii, Okada, & Schuerch, 1993; Kadokawa, Yamamoto, Tagaya, & Chiba, 2001; Kong & Schuerch, 1984; Liang, Franz, Newbury, Lebrilla, & Patten, 2002; Schuerch, 1981).

The Ferrier rearrangement involves the Lewis acid-catalyzed reaction of glycals with a nucleophile, to furnish, via the elimination of the C-3 allylic substituent and rearrangement of the allylic system, 2,3-unsaturated glycosides with a good stereoselectivity (Scheme 1a).

The Ferrier rearrangement has been utilized for the synthesis of O-, S- N- and C-glycosides, and the influence of the reaction conditions and the used Lewis acid on the process has been extensively examined (Ferrier & Hoberg, 2003; Ferrier & Zubkov, 2003). However, the application of this useful reaction for the synthesis of new, biologically active polysaccharides has not been studied so far.

With the goal to synthesize new types of α-amylase enzyme-inhibitory compounds, here we show the extension and utilization of the Ferrier rearrangement for sugar polycondensation.

3,6-Di-O-acetyl-D-glucal (**2**), prepared form D-glucal (**1**) by partial acetylation with 1-acetoxy-benzotriazole, was used as the starting monomer (Scheme 1b). Treatment of **2** with various Lewis acids resulted in the expected polycondensation reaction. As proved by instrumental studies, a high degree of polycondensation was carried out in the presence of Zeolit ZnX during one week, but with $ZnCl_2$ the polycondensation proceeded sufficiently within 24 h.

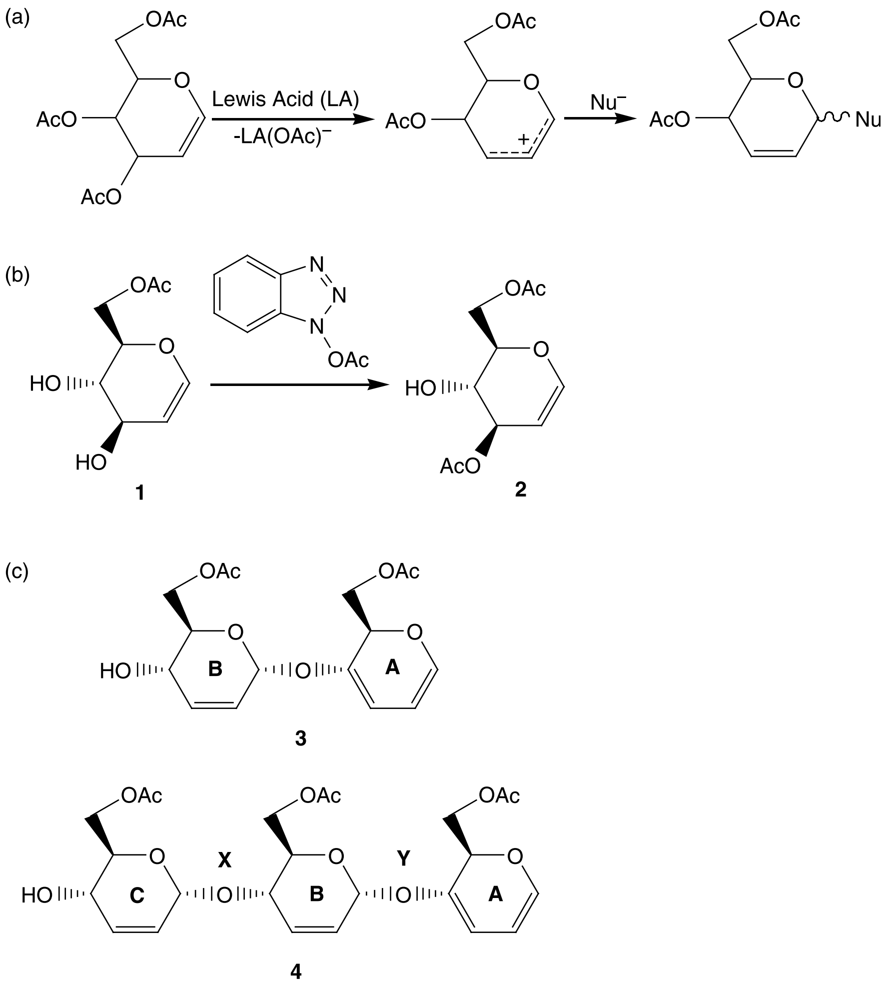
The partial MALDI-TOF MS spectrum of the polymer obtained (Fig. 1) shows the presence of three different series of peaks (a, b and c).

Each of the three series possesses the same mass for the repeating unit (170 Da), i.e., the mass of the expected glycan unit. Oligomers up to $m/z \sim 5500$ (up to 30 repeating

---

* Corresponding authors. Tel.: +52 512900.
E-mail addresses: zsugam@tigris.unideb.hu (M. Zsuga), herczeghp@tigris.unideb.hu (P. Herczegh).

0144-8617/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2005.07.036



Scheme 1. (a) The Ferrier rearrangement, (b) Synthesis of the starting monomer, (c) The structure of the oligomers isolated.

units) could be identified. The mass of series a and c differ by 60 and 42 Da from that of series b, i.e., by the mass of an acetic acid and a ketene unit, respectively. The proposed structures for series **a**, **b** and **c** are also depicted in Fig. 1.

Disaccharide **3** and trisaccharide **4** were isolated and their structures were unambiguously identified by full ^{1}H- ^{13}C assignment (Scheme 1c).

This finding, in good agreement with the MALDI results, gave further evidence for the structure of series a, which was the major polymer series in the product. In the ^{13}C-HSQC spectrum of the polymer three important anomeric CH signals (C_1, B_1 and a minor β isomer) were detected (Fig. 2).

On the basis of the spectra of **4**, one α-glycoside (100%) could be assigned as type X, and the other (20%) as type Y (Scheme 1c). A β-anomeric signal appeared with ca. 10% intensity at 98.3/5.17 ppm revealing a good stereoselectivity of the polyglycosylation.

Based on the experimental results, the proposed mechanism for the polycondensation reaction of glucal **2** is presented in Scheme 2.

According to Scheme 2, polycondensation starts with the delocalized glycosylium cation formed by the Lewis acid-promoted elimination of the acetate anion from **2**, followed by a nucleophilic attack of the 4-OH group of another molecule of **2**. (reaction R1 in Scheme 2). The whole polycondensation proceeds in a similar way, i.e., oligomers with glycosylium cation endgroups react with those carrying nucleophilic OH groups of the 2,3-enoside head groups (reaction R2). The resulting oligomers with 3,6-di-O-acetyl-glucal endgroups (dormant chains) can be again reactivated in the presence of Lewis acids as depicted in Scheme 2 (reaction R3). However, there are two termination steps (reaction R4 and R5) that should be taken into account as proved by the NMR and MALDI studies. The growing glycosylium cation may lose proton to yield 1,3-dienitol chain ends (reaction R4) and simultaneously can accept OH^- ions from the gegenion $LA(OH)^-$ may be formed via the reaction of water impurities ("H_2O") with Lewis acid, to give a hex-2,3-enose chain end (reaction R5).

It is interesting to note that the resulting ion intensities for series a (in the range of n from 3 to 15) can be scaled as Ap^n (where A, p and n are the scaling constant, the extent of polycondensation and the degree of polycondensation, respectively) which is characteristic of the step polymerisations (Flory, 1953).

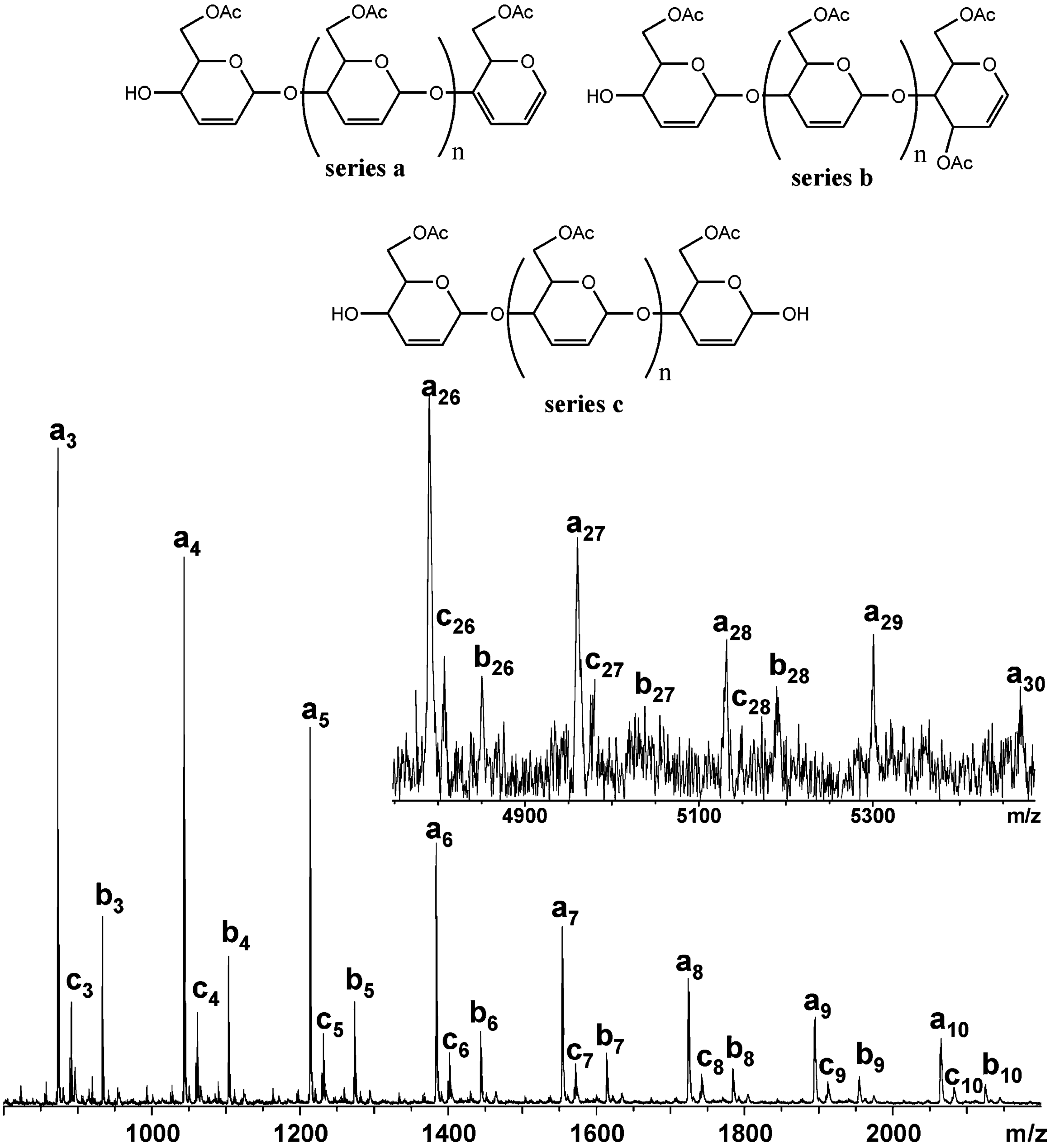


Fig. 1. The partial MALDI TOF MS spectrum of the polymer obtained together with the assignations of the polymer series. The subscript numbers represent the degree of polycondensation. Oligomers are cationized with sodium ions ($[M+Na]^+$).

Using diffusion-ordered NMR (DOSY) (Johnson, 1999) in $CDCl_3$ solution, aggregates with masses up to 30 kDa were observed. Diluting the solution, or using DMSO-d_6 solvent resulted in the disappearance of the species with higher molecular mass. These phenomena can be explained by a unique self-assembly of the oligomers in chloroform that can be avoided by dilution or using a solvent unfavourable for the formation of hydrogen-bonded aggregates.

The polymers obtained can be regarded as 2,3-dideoxy-2, 3-didehydrostarch and -glycogene derivatives. Thus, these compounds are expected to inhibit the active enzymes involved in the biotransformation of such compounds. Indeed, the deacetylated polymer proved to be a semi-competitive inhibitor of the human salivary amylase enzyme with inhibition constants K_{EI} and K_{ESI} of 35.2 and 2.7 mM, respectively.

In summary, the Ferrier-type polycondensation reaction of the simple 3,6-di-O-acetyl-D-glucal monomer furnished unprecedented unsaturated glycans.

Acknowledgements

This work was supported by the Hungarian Research Foundation through grants OTKA T 42512, T 42740, T 37448, T 42567 and the grants NKFP 3/A0036/2002 and RET

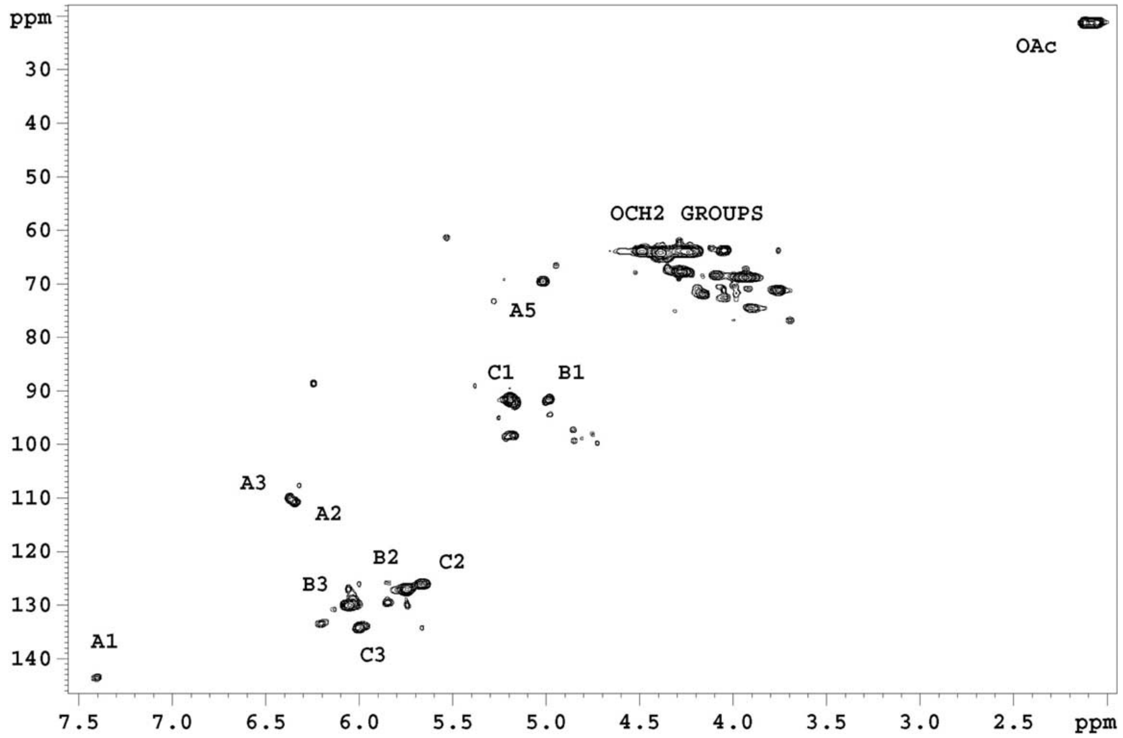
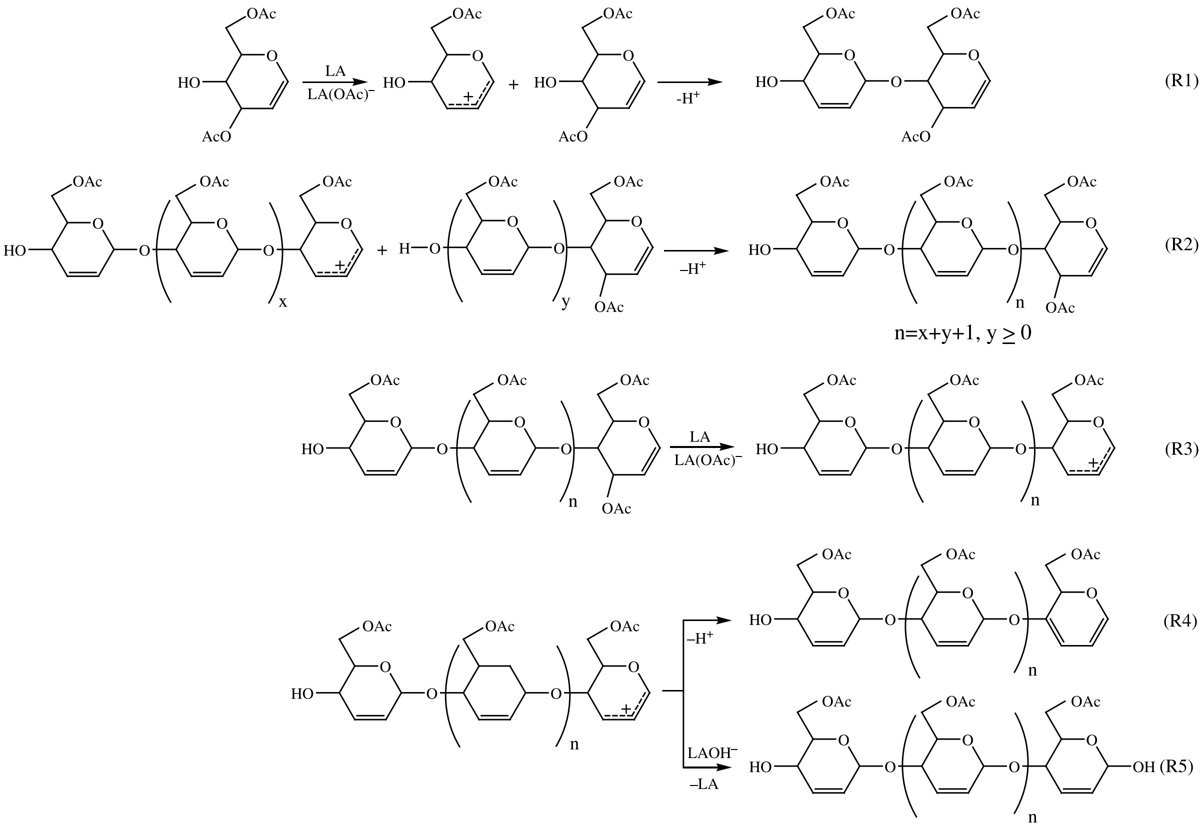


Fig. 2. HSQC spectrum of the polymer. (A, B and C denotes the rings as indicated in Scheme 1b, and the numbers indicate the positions of carbon atoms in the given ring).



Scheme 2. Proposed mechanism for the polycondensation.

006/2004. S.K. thanks for the Bolyai János fellowship. The authors also thank to CELLADAM Ltd., Hungary for providing the BIFLEX III MALDI-TOF MS instrument.

Appendix. Supplementary Material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2005.07.036

References

Ferrier, R. J., & Hoberg, J. O. (2003). *Advances in Carbohydrate Chemistry*, *58*, 55–119.

Ferrier, R. J., & Zubkov, O. A. (2003). *Organic Reactions*, *62*, 569–736.

Flory, P. J. (1953). *Principles of polymer chemistry* (12th ed.), Ch. 8. Ithaca, NY: Cornell University Press.

Good, F. J., & Schuerch, C. (1985). *Macromolecules*, *18*, 595–599.

Ichikawa, H., Kobayashi, K., Sumitomo, H., & Schuerch, C. (1988). *Carbohydrate Research*, *179*, 315–320.

Johnson, C. S. (1999). *Progress in NMR Spectroscopy*, *34*, 203–256. Pelta, M. D., Barjat, H., Morris, G. A., Davis, A. L., & Hammond, S. J. (1999). *Magnetic Resonance in Chemistry*, *36*, 706–714. Groves, P., Rasmussen, M. O., Molero, M. D., Samain, E., Canada, F. J., Driguez, H., et al. (1999). *Glycobiology*, *14*, 451–456.

Kadokawa, J.-I., Yamamoto, M., Tagaya, H., & Chiba, K. (2001). *Carbohydrate Letters*, *4*, 97–101.

Kobayashi, K., Ishii, T., Okada, M., & Schuerch, C. (1993). *Polymer Journal*, *25*.

Kong, F., & Schuerch, C. (1984). *Macromolecules*, *17*, 983–989.

Liang, Y., Franz, A. H., Newbury, C., Lebrilla, C. B., & Patten, T. E. (2002). *Macromolecules*, *35*, 3402–3412.

Schuerch, C. (1981). *Advances in Carbohydrate Chemistry and Biochemistry*, *39*, 157–212.