Isolation, Synthesis and Derivatization of Xylodextrins

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Summary: A series of xylodextrins has been produced by enzymatic or hydrothermal degradation of industrial xylans. For further synthetic use, the oligomers were converted into per-*O*-acetylated xylooligomers which were separated by silica gel chromatography to furnish preparative amounts of xylobiose up to xylopentaose. In a model reaction, selective anomeric deacetylation and treatment with trichloroacetonitrile furnished a xylobiosyl donor, which was converted into the β-methyl glycoside. In addition, methyl β-D-xylopyranoside was transformed into a suitable glycosyl acceptor via tosylation followed by a double displacement reaction at O-4, allowing for further chain elongation and modification at the reducing xylopyranosyl unit.

Keywords: biopolymers; cellulose; renewable resources; Synthesis; xylan

Introduction

Xylans as highly abundant biopolymers in plants constitute a huge reservoir of renewable raw materials which has not been fully exploited until now. Most applications comprise use of xylans as food additives, gelling and thickening agents, adhesives, adsorbants, stabilizers or as pharmaceuticals.[1] Due to a lack of readily available low-molecular weight xylans, manufacture and applications of xylodextrins have not been investigated in detail thus far. Previously, xylooligomers have been obtained by partial acid or enzymatic hydrolysis of hardwood xylans.[2–5] Alternatively, chemical syntheses of defined xylodextrins have been performed, which, however, comprise lengthy protecting schemes and frequently rely on the use of toxic heavy metals for glycoside formation.[6] Recently, a transglycosylation reaction employing a β-D-xylosidase from *Aspergillus* sp. was highly effective to produce labeled xylooligomers up to a DP of 6.[7] Herein we describe a combination of enzymatic or hydrothermal degradation of industrial xylan materials with the chemical coupling of xylosyl donors to a suitable acceptor of methyl β-D-xylopyranoside to allow the gram-scale manufacture of xylodextrin methyl glycosides.

Results and Discussion

For the isolation of xylodextrins two sources were evaluated: the enzymatically degraded xylan preparation Xylo-oligo 95P (from Suntory Ltd., Japan) as well as xylan isolated as a by-product of the viscose process from dialyzed press lye (Lenzing AG, Austria). The xylan mixture from Suntory contained a large amount of xylobiose, and in addition to xylooligomers also cellodextrins were detected, rendering the further isolation of defined oligomers except of xylobiose difficult (Figure 1).

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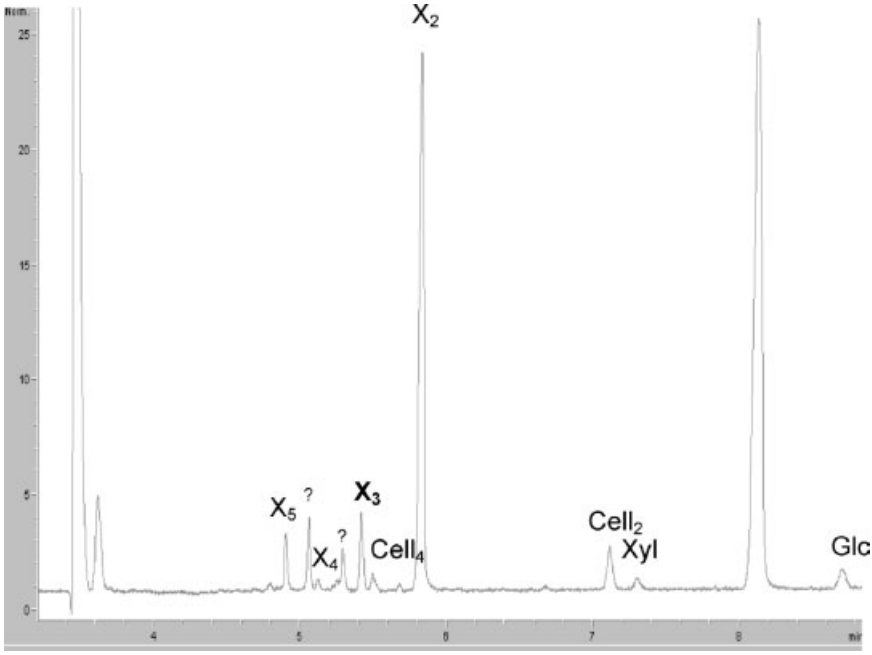

Figure 1.
Electropherogram of the enzyme degraded xylan fractions (Xylo-oligo 95P).

In the second case, beech wood xylan from dialyzed press lye was depolymerized using either a hydrothermal treatment followed by nanofiltration or by enzymatic hydrolysis with endo-1,4-β-xylanase 3.2.1.8.[8] This way, either xylodextrins of nearly equal DP distribution were obtained using the hydrothermal approach (Figure 2),

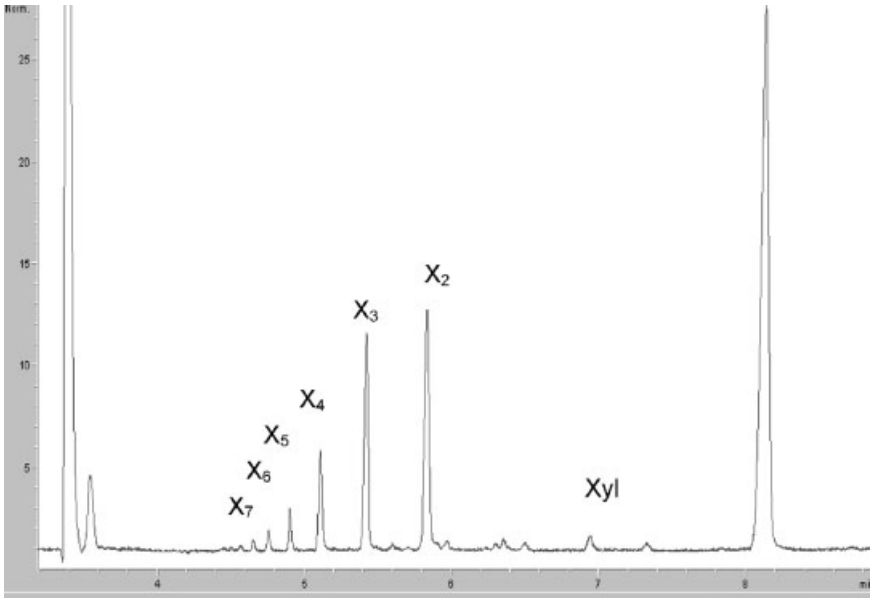

Figure 2.
Electropherogram of the hydrothermally degraded xylan fraction from Lenzing.

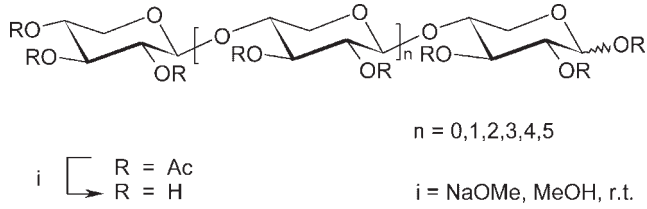

Figure 3.
Isolation and deprotection of per-*O*-acetyl xylodextrins.

whereas mainly xylobiose was generated using the enzymatic degradation. Figure 1 and Figure 2 display the electropherograms of the degraded xylan materials following conversion of the reducing sugars into the corresponding ethyl *p*-aminobenzoates by reductive amination and subsequent separation by capillary zone electrophoresis as reported.[9]

For isolation and synthetic applications the xylan mixtures were subjected to standard *O*-acetylation followed by separation on a preparative silica gel column using a gradient 2:1 → 1:1 *n*-hexane/ethyl acetate. Thus, gram amounts of xylobiose, triose, tetraose and pentaose were obtained in pure form and were fully characterized by NMR spectroscopy. Deprotection of the acetylated oligomers under Zemplén conditions furnished the reducing xylodextrins in high yield (Figure 3). The NMR data of the compounds were in full agreement with previously published spectra. [6]

The per-*O*-acetylated xylooligomers are readily converted into glycosyl donors via selective de-*O*-acetylation at the anomeric center and by subsequent treatment with trichloroacetonitrile under basic conditions trichloroacetimidate donors are furnished. These oligomeric glycosyl donors may be exploited for further coupling to various glycosyl acceptors. In case of xylopyranoside acceptor derivatives, protecting groups have to be introduced, which should allow differentiation between three equatorial hydroxy groups. Previously, regioselective reactions have been performed using isopropylidene acetals or acylating agents in the presence of stannylidene acetals. [10,11] Recently, tosylation has been reported as an approach to selectively protected pentopyranosides. [12] Following the latter procedure, methyl β-D-xylopyranoside **1** was converted in 90% yield into the 2,3,4-tri-*O*-tosyl derivative **2** (Figure 4).

The introduction of three tosyl groups led to inversion from the $^4C_1$-conformation into a $^1C_4$ conformer, as seen from the small values of the vicinal coupling constants $J_{2,3}$ and $J_{3,4}$ (~5.0 Hz). Selective cleavage of the 4-*O*-tosyl group with inversion of configuration was effected by treatment with $NaNO_2$ in DMF at high temperature, which furnished the L-*arabino*-configured methyl α-glycoside **3** present in the $^4C_1$-conformation in 50% yield.

For the regeneration of the *xylo*-configured sugar containing a free hydroxy group at C-4, a second inversion was performed by introduction of a 4-*O*-triflate ester group

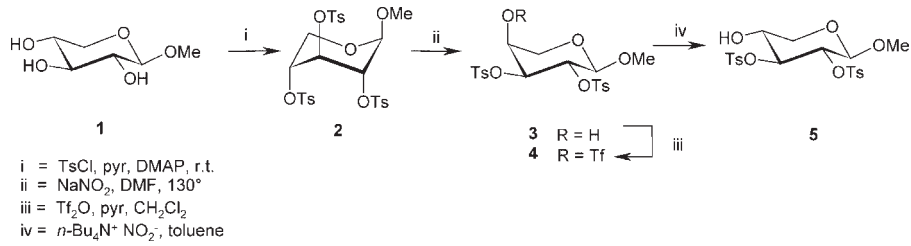

Figure 4.
Synthesis of xylopyranoside acceptor derivative **5**.

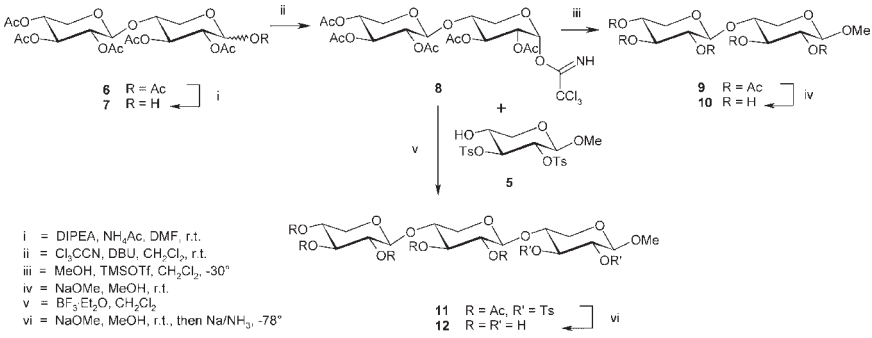

Figure 5.
Di- and trisaccharide synthesis.

(98%) followed by subsequent nucleophilic displacement with tetrabutylammonium nitrite in toluene to afford the glycosyl acceptor derivative **5** in 60% yield.

Glycosylation reactions were performed with the xylobiosyl trichloroacetimidate derivative **8** as model donor. Selective de-*O*-acetylation was achieved in 64% yield by treatment of the peracetylated xylobiose **6** with Hünig-base (DIPEA) / ammonium acetate in DMF. The α-trichloroacetimidate donor **8** was formed by reaction of **7** with trichloroacetonitrile in the presence of DBU under thermodynamic control. Conversion into the β-methyl glycoside **9** in 50% isolated yield was achieved by treatment of donor **8** with methanol in dichloromethane in the presence of TMSO-triflate at -30°. The formation of the β-anomer **9** was deduced from the large value of the coupling constant $J_{1,2}$ (~7.3 Hz) indicating a *trans*-orientation of H-1 and H-2. Minor by-products in the glycosylation step resulted from hydrolysis of the glycosyl donor.

In addition, donor **8** was coupled to the 2,3-di-*O*-tosyl derivative **6** using $BF_3 \cdot Et_2O$ as promoter. Again, only formation of the

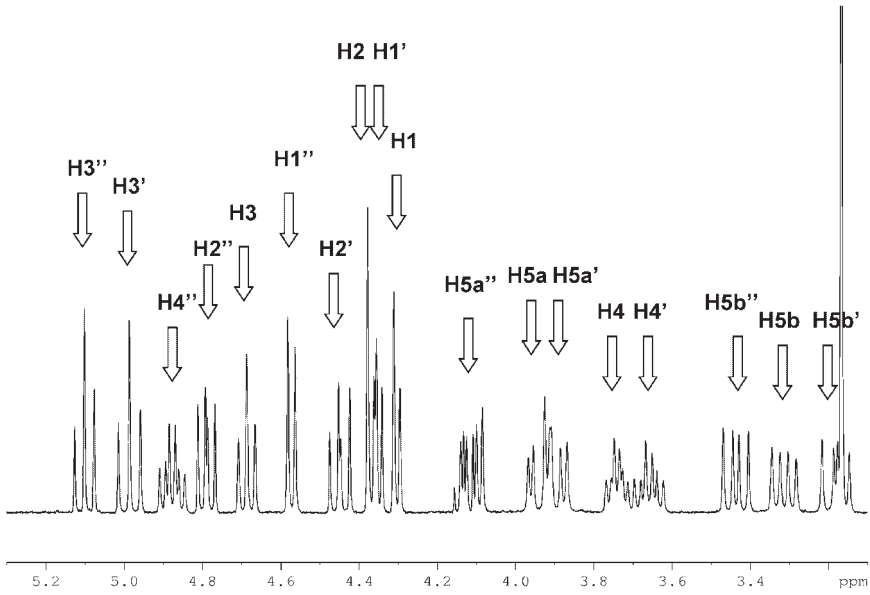

Figure 6.
300 MHz ^{1}H NMR spectrum ($CDCl_3$) of trisaccharide derivative **11**.

β-linked trisaccharide derivative **11** was observed in a similar yield as compared to the methyl glycoside **9** (Figure 5).

In the ^{1}H NMR spectrum of trisaccharide **11** all signals were well resolved and could be fully assigned (Figure 6). The tosyl groups at the reducing xylopyranosyl unit may be further transformed into other functional groups via nucleophilic substitution reactions. Deprotection of the trisaccharide was accomplished in two steps by Zemplén deacetylation followed by treatment with sodium in liquid ammonia which furnished the methyl xylotrioside **12** in 64% yield. NMR data of **12** were in full agreement with published data. [6]

Conclusion

In conclusion, the availability of numerous xylooligomers in pure form from degraded xylan fractions allows the recovery and synthesis of versatile xylodextrin derivatives, thereby considerably minimizing the synthetic steps encountered in stepwise or blockwise assembly of xylooligosaccharides.

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