Chromophores from hexeneuronic acids (HexA): synthesis of model compounds and primary degradation intermediates

Thomas Rosenau · Antje Potthast · Nele Sophie Zwirchmayr · Takashi Hosoya · Hubert Hettegger · Markus Bacher · Karin Krainz · Yuko Yoneda · Thomas Dietz

Received: 9 April 2017 / Accepted: 1 July 2017 / Published online: 13 July 2017
© The Author(s) 2017. This article is an open access publication

Abstract Hexeneuronic acid (HexA) is formed under pulping conditions from 4-*O*-methyl-glucuronic acid residues in xylans by methanol elimination. It is usually removed by an acidic washing treatment (A-stage) within the pulp bleaching sequence. Hexeneuronic acid has long been recognized as a source of color generation in pulps, but the chemical structure of the actual chromophoric compounds remained elusive. We report the synthesis of isotopically (^{13}C) labeled HexA model units carrying a label at any of the six carbon atoms. Confirming pertinent literature accounts, it is shown that HexA forms three primary

degradation intermediates, 2-furancarboxylic acid, 5-formyl-2-furancarboxylic acid, and formic acid, under mildly acidic conditions, and their formation mechanism is discussed. 2-Furancarboxylic acid is demonstrated to be deformylation product of 5-formyl-2-furancarboxylic acid. The three primary intermediates are colorless and do not represent chromophores themselves. Their mixture, upon thermal or acidic treatment, gives rise to the same chromophores that are also directly formed from HexA.

Keywords Cellulose · Pulp · Chromophores · Hexeneuronic acids · Bleaching · A-stage · Furan · Furancarboxylic acid · Ladder-type oligomers

Chromophores in cellulosic materials. Part XV.

This work is dedicated to Dr. Hans-Ulrich Suess, formerly Degussa and Degussa-Evonik, who devoted his whole life to the understanding of chromophore formation in cellulosic pulps and bleaching phenomena.

T. Rosenau (✉) · A. Potthast · N. S. Zwirchmayr · T. Hosoya · H. Hettegger · M. Bacher
Division of Chemistry of Renewable Resources,
Department of Chemistry, BOKU University Vienna,
Muthgasse 18, 1190 Vienna, Austria
e-mail: thomas.rosenau@boku.ac.at

K. Krainz
Air Liquid GmbH, Sendnergasse 30, 2320 Schwechat,
Austria

Y. Yoneda
College of Agriculture, Shizuoka University, Ohya 836,
Suruga-ku, Shizuoka 422-8529, Japan

T. Dietz
Evonik-Degussa, Rodenbacher Chaussee 4,
63457 Hanau-Wolfgang, Germany

T. Rosenau
Johan Gadolin Process Chemistry Centre, Åbo Akademi
University, Porthansgatan 3, 20500 Åbo/Turku, Finland

Introduction

The CRI method (chromophore release and identification) opened the way to isolate and identify well-defined chromophoric structures from cellulosic matrices, despite their very low concentration (Rosenau et al. 2004). This technique has been applied to different cellulosic materials, including cellulose I substrates (pulp, (Rosenau et al. 2007, 2008) bacterial cellulose, (Rosenau et al. 2014) cotton (Rosenau et al. 2011), cellulose II substrates (regenerated celluloses such as rayon or Lyocell fibers), (Adorjan et al. 2005; Rosenau et al. 2005) and cellulose derivatives (cellulose 2,5-acetates, cellulose 3-acetates) (Rosenau et al. 2005). In each case, different numbers of compounds (between 3 and 12 individual compounds) at different concentrations (between 4 and 42 ppm for the chromophore mixture) have been isolated. Making individual chromophoric structures known and rendering the compounds accessible to chemical, analytical, and bleaching studies can be seen as the main benefit of the method. With this basic knowledge at hand, industrial bleaching sequences can be optimized, discoloration treatments followed more easily, and destruction of chromophores optimized while at the same time keeping chemical usage and energy costs down, and accounts of this approach have been published.

So far, the CRI method has been applied to lignin-free—or at least lignin-poor—cellulosic materials as higher content of lignin would overwhelm the separation and identification capability of the technique (Korntner et al. 2015). The chromophores isolated so far thus resulted exclusively from cellulose, and their formation occurred either by oxidative “aging” (Potthast et al. 2005) with follow-up fragmentations/condensations or through side reactions of cellulose processing (Potthast et al. 2009), such as upon xanthogenation in rayon manufacture, dissolution in *N*-methylmorpholine-*N*-oxide in Lyocell production, or acetylation/deacetylation in cellulose acetate

synthesis or acetic acid- based solvents (Potthast et al. 2002). However, since the CRI approach is quite general, it can not only be used to address cellulose-derived chromophores, but aromatic and quinoid chromophores in polysaccharide matrices in general.

One of the most important of such non-cellulose-derived chromophore sources is hexeneuronic acid (HexA), the term being used as shorthand for the 4-deoxy- β -L-*threo*-hex-4-enopyranosiduronic acid moiety. Within the series on chromophores in cellulosic materials, the present report and the subsequent parts will address the issue of chromophores from HexA, their formation mechanism, and their chemical behavior under standard bleaching conditions. As it was in the case of the cellulose-derived chromophores, the prime challenge was to identify the structures of the individual chromophoric compounds. This was done again with the help of model compounds and by comparison to independently synthesized, authentic samples. With the knowledge of the chemical structures at hand, the second step, a study of the compounds’ destruction in bleaching attempts, is much facilitated as compared to situations when just general or vague structural elements are known or suspected.

Early research on the chemical behavior of hemi-celluloses during the pulping process by Clayton suggested the formation of 4-deoxy- β -L-*threo*-hex-4-enopyranosiduronic acid moieties taking place during alkaline treatment of wood chips for delignification at temperatures of up to 170 °C (Clayton 1963; Chakar et al. 2000). The proposed mechanism was β -elimination of methanol taking place at the 4-*O*-methyl-D-glucuronic acid residues of xylans, resulting in HexA (see Scheme 1). The resulting double bond is always placed regioselectively towards C-5, but not C-3, to enable the conjugated α,β -unsaturated acid structure.

These results of early analytical studies (Clayton 1963) were further affirmed by Johansson and

Scheme 1 Alkali-catalyzed formation of HexA from glucuronoxylan by β -elimination of methanol during pulping. (Chakar et al. 2000)

Samuelsson using dimeric model compounds (Johansson and Samuelson 1977). Acid treatments of HexA-containing pulps caused degradation of the proposed unsaturated uronic acids and only xylans and “tar” were found in the hydrolysate (Chakar et al. 2000; Johansson and Samuelson 1977; Teleman et al. 1995; Shimizu 1981). The application of a highly hemicellulose-specific enzymatic hydrolysis method to kraft pulps by Teleman et al. finally allowed definite identification of these acidic side groups on xylan (Teleman et al. 1995). After enzymatic degradation, acidic oligosaccharides were separated from uncharged components by anion exchange, further fractionated by SEC, and determined to be HexA mainly by NMR experiments. The knowledge on the formation of HexA and its suspected role in chromophore formation lead to an increase in the research efforts regarding its degradation. The bleaching research focused increasingly on the xylan carbohydrate fraction of wood and pulp, the origin of HexA. The contribution of HexA to decreased brightness in pulps, viz. its large influence on the kappa number, and its behavior during acid hydrolyses was thoroughly established (Li and Gellerstedt 1997; Clavijo et al. 2012). Acidic conditions, e.g. at 130 °C for 2–3 h with 0.05 M H₂SO₄, (Johansson and Samuelson 1977) or at 80–140 °C and pH 3.0–3.5 for 2–5 h, (Chakar et al. 2000; Clavijo et al. 2012) allowed removal of the HexA side groups, but also caused occurrence of furanoid primary products, e.g. 2-furancarboxylic acid and 5-formyl-2-furancarboxylic acid, which gave rise to unidentified chromophoric compounds (Clavijo et al. 2012; Teleman et al. 1996). In the following, the mechanism of HexA degradation and the structure of the resulting chromophores will be addressed in detail.

Materials and methods

General

Commercial chemicals were of the highest grade available and were used without further purification. Reagent grade solvents were used for all extractions and workup procedures. Distilled water was used for all aqueous extractions and for all aqueous solutions. *n*-Hexane, diethyl ether, ethyl acetate, and petroleum ether used in chromatography were distilled before

use. All reactions involving non-aqueous conditions were conducted in oven-dried (140 °C, overnight) or flame-dried glassware under an inert argon or nitrogen atmosphere. TLC was performed using Merck silica gel 60 F₂₅₄ pre-coated glass plates. Flash chromatography was performed using Baker silica gel (40 µm particle size). All products were purified to homogeneity by TLC/GC–MS analysis. The use of brine refers to saturated aqueous NaCl solution. All given yields refer to isolated, pure products. ¹³C-Labeled starting compounds were purchased from Omicron Biochemicals, Inc., South Bend, IN, USA, and from Euriso-Top GmbH, Saarbrücken, Germany.

NMR spectra were recorded at 400.13 MHz for ¹H and at 100.62 MHz for ¹³C NMR at room temperature, in the perdeuterated solvents given. Chemical shifts, relative to tetramethylsilane (TMS) as the internal standard, are given in δ ppm values, and coupling constants in Hz. ¹³C peaks were assigned by means of high-resolved 1D-¹³C, APT, HSQC and HMBC spectra. Through experiments without ¹H-decoupling (GD-mode), the complete set of geminal and vicinal homonuclear (*J*_{H,H} and *J*_{C,C}) as well as heteronuclear (*J*_{C,H}) coupling constants was obtained for the 4-deoxy-β-*L*-threo-hex-4-enopyranosiduronic acid moiety by combination of data from the selectively ¹³C-labeled compounds.

GC–MS analysis was carried out on an Agilent 6890 N/5975B instrument in the EI (70 eV) ionization mode. Melting points, determined on a Kofler-type micro hot stage with Reichert-Biovar microscope, are uncorrected. Elemental analyses were performed by the Microanalytical Laboratory at the University of Vienna. All compounds showed satisfactory microanalytical data within the limits of 0.3%.

Synthesis of the ¹³C-labeled HexA model compounds

The following protocols are presented by means of the non-labeled compounds, with methyl 4-deoxy-β-*L*-threo-hex-4-enopyranosiduronic acid (**1**, see Scheme 2) being the target. The same synthesis sequence was repeated six times for the six selectively ¹³C-labeled compounds: methyl 1-¹³C-4-deoxy-β-*L*-threo-hex-4-enopyranosiduronic acid (**1a**), methyl 2-¹³C-4-deoxy-β-*L*-threo-hex-4-enopyranosiduronic acid (**1b**), methyl 3-¹³C-4-deoxy-β-*L*-threo-hex-4-enopyranosiduronic acid (**1c**), methyl 4-¹³C-4-deoxy-β-*L*-threo-hex-4-

i) Ac_2O / I_2 , ii) THF/ H_2O (v/v=9/1), 88 - 92% from **2**, iii) SnCl_4 , CH_2Cl_2 ,
 iv) $\text{SnCl}_4/\text{TMS-OMe}$ / CH_2Cl_2 , then NaHCO_3 , then AcOH , 72-79% from **4**,
 v) $\text{TMS-CHN}_2/\text{MeOH}$, CH_2Cl_2 , vi) $\text{Ac}_2\text{O}/\text{Py/DBU}$, 79-83% from **6**,
 vii) $\text{LiOH}/\text{H}_2\text{O}/\text{THF}$, 92-94%

Scheme 2 Reaction sequence towards HexA model compound methyl 4-deoxy-β-L-threo-hex-4-enopyranosiduronic acid (**1**) starting from D-glucuronic acid

enopyranosiduronic acid (**1d**), methyl 5- ^{13}C -4-deoxy-β-L-threo-hex-4-enopyranosiduronic acid (**1e**), and methyl 6- ^{13}C -4-deoxy-β-L-threo-hex-4-enopyranosiduronic acid (**1f**), starting from the corresponding ^{13}C -labeled D-glucuronic acids. The synthesis followed an optimized synthesis path towards non-labeled model compound **1**, based on a path previously developed in our group (Tot et al. 2008). This way, the syntheses were performed seven times, i.e. for the seven different isotopomers, and the yield of each step is given as the range between the experiment with the lowest and the highest yield. Yield differences between the runs with different isotopomers were generally below 7%, in most cases below 4%.

D-Glucuronic acid (**2**, $M = 194.1$ g/mol, 5.0 g, 25.76 mmol) was suspended in acetic anhydride (75 ml) and stirred at 0 °C for 15 min. Iodine (350 mg, 1.65 mmol) was added and stirring was continued for 2 h, keeping the temperature strictly below 5 °C by efficient ice/water bath cooling. The mixture was allowed to reach r.t., and excess acetic anhydride was removed *in vacuo* at r.t. The residue was diluted with dichloromethane (120 ml), washed three times with cold aqueous $\text{Na}_2\text{S}_2\text{O}_3$ (1 M, 80 ml each), dried over MgSO_4 , and filtered. After removal of the solvent under reduced pressure, the crude peracetylated mixed anhydride **3** was used directly in the next step

without further purification. TLC: dichloromethane/methanol = 9:1, $R_f = 0.24$. ^1H NMR (CDCl_3): δ 5.82 (d, 1H, $J_{1,2} = 6.9$, H-1), 5.39 (t, 1H, $J = 9.0$, H-4), 5.30 (t, 1H, $J = 8.3$, H-3), 5.13 (dd, 1H, H-2), 4.33 (d, 1H, H-5), 2.29 (s, 3H, Me in anhydride), 2.15, 2.06, 2.05, 2.04 (4 s, 4 × 3H, acetoxy).

Compound **3** ($M = 404.3$ g/mol) was dissolved in a mixture of THF and water (50 ml, v/v = 9:1) and stirred overnight. MgSO_4 was added to bind the water present, and additional 50 mL of THF was added and the slurry was filtered. The solvent was removed under reduced pressure to give peracetylated acid **4** (1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranosiduronic acid) which was again used without further purification (91%, 88–92% for **4a–4f**). TLC: dichloromethane/methanol = 3:1, $R_f = 0.55$. ^1H NMR (CDCl_3): δ 5.80 (d, 1H, $J_{1,2} = 7.5$, H-1), 5.31 (m, 2H, H-3 and H-4), 5.15 (m, 1H, $J_{2,3} = 9.0$, H-2), 4.26 (m, 1H, $J_{4,5} = 9.31$, H-5), 2.13, 2.06, 2.05, 2.04 (4 s, 4 × 3H, acetoxy).

Acid **4** ($M = 362.3$ g/mol, 8.49 g, 23.43 mmol) was dissolved in dry dichloromethane (100 ml) under argon. SnCl_4 (2.95 ml, 1.1 eq.) was added during 10 min and the reaction mixture was stirred overnight at r.t. Completion of the lactone formation was checked by TLC control: saturated aqueous NaHCO_3 solution (5 ml) was added to a 5 ml aliquot and the mixture was vigorously stirred for 30 min to guarantee intimate mixing of the two phases. The resulting

viscous white emulsion was filtered under vacuum through a 5 mm layer of Celite®. A filtrate of two phases resulted, of which the aqueous layer was discarded and the organic layer washed with saturated NaHCO₃ solution, dried over MgSO₄, and filtered. The solvent was removed *in vacuo* and the remainder, the crude lactone **5**, taken for analysis. TLC: hexane/ethyl acetate = 1:3, R_f = 0.80, m.p. 72–74 °C. ¹H NMR (CDCl₃): δ 5.92 (s, 1H, H-1), 4.96 (s, 1H, H-3), 4.82 (s, 1H, H-4), 4.78 (s, 1H, H-2), 4.60 (t, 1H, H-5), 2.19 (s, 6H, 2-*O*-acetoxy, 4-*O*-acetoxy), 2.10 (s, 3H, 3-*O*-acetoxy).

To the main reaction mixture, methoxytrimethylsilyl-lane (TMSOMe, 1.05 eq., 25 mmol, 3.15 ml) was added, and the reaction mixture was left stirring overnight. Saturated aqueous NaHCO₃ solution (50 ml) was added and the mixture vigorously stirred for 30 min to guarantee intimate mixing of the two phases. The resulting viscous white emulsion was left standing for 30 min and glacial acetic acid was added under CO₂ evolution until no more gas was released and two clear phases resulted. The phases were separated and the aqueous phase was extracted with dichloromethane (3 × 20 mL). The combined organic phases were washed with brine (10 ml), dried over MgSO₄, and filtered. The reaction mixture was concentrated and the crude product was purified by silica gel chromatography using chloroform/ethanol/water = 5:2:0.1 as eluent, affording 2,3,4-tri-*O*-acetyl-1-*O*-methyl-α-D-glucopyranosiduronic acid (**6**) in 77% yield (72–79% for **6a–6f**). TLC: CHCl₃/EtOH/H₂O = 5:2:0.1, R_f = 0.31. ¹H NMR (CDCl₃): δ 8.4 (br s, 1H, COOH), 5.53 (dd, 1H, $J_{2,3}$ = 10.0, $J_{3,4}$ = 9.5, H-3), 5.22 (dd, 1H, $J_{4,5}$ = 10.1, H-4), 5.06 (d, 1H, $J_{1,2}$ = 3.5, H-1), 4.92 (dd, 1H, H-2), 4.32 (d, 1H, H-5), 3.46 (s, 3H, OCH₃), 2.08, 2.04, 2.02 (3 s, 3 × 3H, acetoxy).

Peracetylated acid **6** (324.28 g/mol, 5.85 g, 18.04 mmol) was dissolved in dichloromethane (150 ml). Methanol (5 ml) and trimethylsilyldiazomethane (TMSDAM, 1 M in diethyl ether, 21.65 ml, 1.2 eq.) were added. Stirring was continued for 1 h and the mixture was refluxed for 5 min. A 5 ml aliquot was taken, the solvent removed by evaporation and the remainder, the corresponding methyl ester **7**, analyzed by NMR. Methyl 2,3,4-tri-*O*-acetyl-1-*O*-methyl-α-D-glucopyranosiduronate (**7**): TLC: CHCl₃/EtOH = 5:2, R_f = 0.54. ¹H NMR (CDCl₃): δ 5.51 (dd, 1H, $J_{2,3}$ = 9.9, $J_{3,4}$ = 8.5, H-3), 5.08 (dd, 1H,

$J_{4,5}$ = 10.3, H-4), 5.16 (d, 1H, $J_{1,2}$ = 4.4, H-1), 5.00 (dd, 1H, H-2), 4.28 (d, 1H, H-5), 3.46 (s, 3H, OCH₃), 3.24 (s, 3H, COOMe), 2.08, 2.04, 2.02 (3 s, 3 × 3H, acetoxy).

After concentrating the main reaction mixture to a volume of about 50 mL, a mixture of acetic anhydride and pyridine (v/v = 1:1, 100 ml) was added, along with DBU (3 ml) as a catalyst. The mixture was stirred for 72 h at r.t. The solvent was removed *in vacuo*, the remainder diluted with EtOAc (50 ml), washed with brine (2 × 10 ml), and dried over MgSO₄. The solvent was evaporated and the crude product was purified by column chromatography on silica gel (hexane/ethyl acetate, v/v = 1:5) to give **8**, the acetyl-protected methyl ester of the target product (83%, 79–83% for **8a–8f**). TLC: EtOAc/MeOH = 3:1, R_f = 0.54. ¹H NMR (CD₃OD): δ 6.04 (d, 1H, $J_{3,4}$ = 3.6, H-4), 5.57 (dd, 1H, $J_{2,3}$ = 8.6, H-3), 5.22 (d, 1H, $J_{1,2}$ = 3.0, H-1), 5.08 (dd, 1H, H-2), 3.52 (s, 3H, OCH₃), 3.28 (s, 3H, COOMe), 2.07, 2.06 (2 s, 2 × 3H, acetoxy).

Methyl ester **8** (288.25 g/mol, 4.32 g, 14.97 mmol) was suspended in 50 ml of aqueous LiOH solution (0.1 M in H₂O/THF, v/v = 2:1) at 0 °C (ice bath) and was stirred for 30 min. The reaction mixture was diluted with 50 mL of water and acidified to pH 2 with ion exchange resin Dowex 50 (H⁺ form). The ion exchanger was removed by filtration, the mixture was concentrated *in vacuo* at r.t. and the precipitate lyophilized to dryness, affording **1** (190.15 g/mol) as colorless amorphous solid which was purified by recrystallization from methanol (2.62 g, 13.77 mmol, yield 92%, 92–94% for **1a–1f**). TLC: MeOH/CH₂Cl₂/H₂O = 5:10:1, R_f = 0.6.

The complete set of NMR data of methyl 4-deoxy-β-L-*threo*-hex-4-enopyranosiduronic acid (**1**) and its corresponding ¹³C-labeled isotopomers (**1a–1f**), including the homonuclear (H,H and C,C) and heteronuclear (C,H) coupling constants, are summarized in Table 1 (¹H domain) and Table 2 (¹³C domain).

Primary degradation products of the HexA model compounds—synthesis of 5-formyl-2-furancarboxylic acid (**9** and isotopomers **9a–9f**) and 2-furancarboxylic acid (**10** and isotopomers **10b–10f**)

Methyl 4-deoxy-β-L-*threo*-hex-4-enopyranosiduronic acid (**1**, 190 mg, 1 mmol), or its isotopomers **1a–1f**, were dissolved in 10 ml of phosphate buffer

Table 1 ^1H NMR (400 MHz, D_2O) data of methyl 4-deoxy- β -L-*threo*-hex-4-enopyranosiduronic acid (**1**) and its ^{13}C -labeled isotopomers (**1a–1f**) including chemical shifts (data in ppm) and the homonuclear (H,H) and heteronuclear (C,H) coupling constants (data in Hz)

Atom	1	1a	1b	1c	1d	1e	1f
H-1	4.95, d, $^3J_{\text{H1,H2}} = 2.8$	4.95, dd, $^1J_{\text{C1,H1}} = 164.8$	4.95, dd, $^2J_{\text{C2,H1}} = 0.5$	4.95, dd, $^3J_{\text{C3,H1}} = 6.1$	4.95, d, $^3J_{\text{H1,H2}} = 2.8$	4.95, d, $^3J_{\text{H1,H2}} = 2.8$	4.95, d, $^3J_{\text{H1,H2}} = 2.8$
H-2	3.72, dd, $^3J_{\text{H1,H2}} = 2.8$, $^3J_{\text{H2,H3}} = 7.6$	3.72, ddd, $^2J_{\text{C1,H2}} = 0.3$	3.72, ddd, $^1J_{\text{C2,H2}} = 127.9$	3.74, m, $^2J_{\text{C3,H2}} = 4.1$	3.74, dt, $^3J_{\text{C4,H2}} = 2.8$	3.72, dd	3.72, dd
H-3	4.21, dd, $^3J_{\text{H2,H3}} = 7.6$, $^3J_{\text{H3,H4}} = 2.8$	4.21, ddd, $^3J_{\text{C1,H3}} = 1.8$	4.20, dt, $^2J_{\text{C2,H3}} = 2.8$	4.20, ddd, $^1J_{\text{C3,H3}} = 125.1$	4.20, ddd, $^2J_{\text{C4,H3}} = 6.0$	4.20, dd, $^3J_{\text{C5,H3}} < 0.1$	4.21, dd
H-4	5.95, d, $^3J_{\text{H3,H4}} = 2.8$	5.95, d	5.95, dd, $^3J_{\text{C2,H4}} = 6.4$	5.95, dd, $^2J_{\text{C3,H4}} = 4.5$	5.96, dd, $^1J_{\text{C4,H4}} = 169.4$	5.95, dd, $^2J_{\text{C5,H4}} = 11.3$	5.95, dd, $^3J_{\text{C6,H4}} = 5.3$
OMe	3.44, s	3.44, d, $^3J_{\text{C1,H}} = 4.6$	3.44, s	3.44, s	3.42, s	3.44, s	3.44, s

(pH = 4.5). The mixture was stirred under argon and under exclusion of light (vessel wrapped in aluminum foil) for about 19 h at r.t., until TLC control ($\text{MeOH}/\text{CH}_2\text{Cl}_2/\text{H}_2\text{O} = 5:10:1$, $R_f = 0.60$) showed that the starting material was consumed completely. Longer reaction times will shift the ratio between the products **9** and **10** in favor of the latter (18% of **9** and 75% of **10** after 72 h). Powdered Na_2SO_4 was added until the mixture was saturated and no more salt dissolved. The mixture was extracted with dichloromethane (3×20 ml), the extracts were combined, and the solvent removed *in vacuo* at r.t. The remaining waxy and slightly yellow remainder was chromatographed on silica gel using chloroform/ethanol/water = 5:2:0.1 as eluent, providing 5-formyl-2-furancarboxylic acid (**9**) as the first main fraction (140.1 g/mol, 102.25 mg, 73%, 56–82% for **9a–9f**) and 2-furancarboxylic acid (**10**) as the second main fraction (112.1 g/mol, 25.8 mg, 23%, 12–41% for **10b–10f**).

The complete sets of NMR data of 5-formyl-2-furancarboxylic acid (**9**) and 2-furancarboxylic acid (**10**) and their corresponding ^{13}C -labeled isotopomers (**9a–9f** and **10b–10f**), including the homonuclear (H,H and C,C) and heteronuclear (C,H) coupling constants, are summarized in Tables 3 and 6 (^1H domain) as well as Tables 4, 5, 7 and 8 (^{13}C domain), respectively.

5-Formyl-2-furancarboxylic acid and its isotopomers (**9**, **9a–9f**)

Note that the numbering for compound **9** and its isotopomers (see formula inset) does not correspond to the IUPAC numbering, but was derived from parent compound **1** for a better illustration of the mechanistic and structural dependence.

2-Furancarboxylic acid and its isotopomers (**10**, **10b–10f**)

Note that the numbering for compound **10** and its isotopomers (see formula inset) does not correspond to the IUPAC numbering, but was derived from parent compound **1** to better illustrate the mechanistic and structural dependence.

Formic acid- ^{13}C (**11a**) was obtained upon degradation of HexA model compound **1a** via intermediate **9a** besides non-labeled 2-furancarboxylic acid **10** (therefore no compound “**10a**” is listed as the ^{13}C -

Table 2 ^{13}C NMR (100 MHz, D_2O) data of methyl 4-deoxy- β -L-*threo*-hex-4-enopyranosiduronic acid (**1**) and its ^{13}C -labeled isotopomers (**1a–1f**) including chemical shifts (data in ppm) and the homonuclear (C,C) couplings (data in Hz)

Atom	1	1a	1b	1c	1d	1e	1f
C-1	100.5	100.5*	100.6, d, $^1J_{\text{C,C}} = 38.5$	100.5, d, $^2J_{\text{C,C}} = 2.4$	100.5, d, $^3J_{\text{C,C}} = 0.3$	100.5, d, $^2J_{\text{C,C}} = 2.5$	100.5, d, $^3J_{\text{C,C}} = 3.6$
C-2	69.6	69.8, d, $^1J_{\text{C,C}} = 38.5$	69.7*	69.7, d, $^1J_{\text{C,C}} = 42.6$	69.6, d, $^2J_{\text{C,C}} = 2.6$	69.6, d, $^3J_{\text{C,C}} < 0.1$	69.6
C-3	65.6	65.6, d, $^2J_{\text{C,C}} = 2.4$	65.7, d, $^1J_{\text{C,C}} = 42.6$	65.6*	65.6, d, $^1J_{\text{C,C}} = 40.6$	65.6, d, $^2J_{\text{C,C}} = 3.1$	65.6, d, $^3J_{\text{C,C}} = 3.5$
C-4	111.6	111.6, d, $^3J_{\text{C,C}} = 0.3$	111.6, d, $^2J_{\text{C,C}} = 2.6$	111.6, d, $^1J_{\text{C,C}} = 40.6$	111.7*	111.8, d, $^1J_{\text{C,C}} = 54.2$	111.5, d, $^2J_{\text{C,C}} = 0.4$
C-5	141.1	141.0, d, $^2J_{\text{C,C}} = 2.5$	141.1, d, $^3J_{\text{C,C}} < 0.1$	141.1, d, $^2J_{\text{C,C}} = 3.1$	141.4, d, $^1J_{\text{C,C}} = 54.2$	141.1*	141.2, d, $^1J_{\text{C,C}} = 74.0$
COOH	166.3	166.3, d, $^3J_{\text{C,C}} = 3.5$	166.2	166.6, d, $^3J_{\text{C,C}} = 3.6$	166.6, d, $^2J_{\text{C,C}} = 0.4$	166.1, d, $^1J_{\text{C,C}} = 74.0$	166.5*
OMe	56.8	56.8, d, $^2J_{\text{C,C}} = 2.5$	56.8, d, $^3J_{\text{C,C}} = 0.3$	56.8	56.8	56.8	56.8

The heteronuclear coupling constants (C, H) are listed in Table 1

* >99.9% ^{13}C

label at C1 is cleaved off as formic acid in this reaction).

^1H NMR ($\text{DMSO}-d_6$): δ 8.01, $^1J_{\text{C,H}} = 215.3$. ^{13}C NMR ($\text{DMSO}-d_6$): δ 163.76. ^1H NMR (D_2O): δ 8.24, $^1J_{\text{C,H}} = 219.2$. ^{13}C NMR (D_2O): δ 168.3.

Results and discussion

Synthesis of the HexA model compound

HexA model compound **1** represents the 4-deoxy- β -L-*threo*-hex-4-enopyranosiduronic acid side-chain moiety in xylans, with the backbone chain being mimicked by the methyl aglycon. Under the harsh conditions of pulping, these residues are formed by methanol elimination from the 4-*O*-methylglucuronic acids that are 1 $\rightarrow$ 2- α -glycosidically linked to the xylopyranan. The synthesis of the model compound was thoroughly optimized with regard to yield and recycling of side products in order to minimize the loss of rather costly ^{13}C -labeled material in the subsequent synthesis of the six isotopomers **1a–1f**. The synthesis scheme, in principle, followed a sequence that had been previously developed in our group (Tot et al. 2008), with some improvements to work more efficiently in the case of the labeled compounds. In particular, we tried to employ one-pot procedures

whenever possible and reduce the number of (chromatographic) purifications between the steps to minimize inevitable compound losses.

The synthesis towards methyl 4-deoxy- β -L-*threo*-hex-4-enopyranosiduronic acid (**1**) comprised a seven step sequence starting from readily accessible D-glucopyranosiduronic acid (see Scheme 2). This starting material is also commercially available, albeit quite costly, with a mono- ^{13}C -label at each of the six carbon atoms. In the first step, the glucopyranosiduronic acid **2** was peracetylated with acetic anhydride/iodine. Under these conditions, also the carboxylic acid function was acetylated so that a mixed anhydride **3** was formed, which was readily cleaved by water into the free acid **4**. Although alternative acetylation conditions, such as standard amine catalysis, avoid the additional step of anhydride formation and cleavage, they are still far inferior by giving lactone and furanoid by-products that are completely absent upon iodine catalysis.

The following two transformations were the key steps in the sequence, both carried out as one-pot reaction and promoted by the same reagent, namely SnCl_4 . Treatment of acid **4** with SnCl_4 afforded the bicyclic, β -configured 1,6-lactone **5** under pyranoside ring inversion. Addition of methanol and methoxytrimethylsilane opened this lactone again in a fully regioselective manner: the nucleophilic attack

Table 3 ^1H NMR (400 MHz, DMSO-d_6) data of 5-formyl-2-furancarboxylic acid (**9**) and its ^{13}C -labeled isotopomers (**9a–9f**) including chemical shifts (data in ppm) and the homonuclear (H,H) and heteronuclear (C,H) coupling constants (data in Hz)

	9	9a	9b	9c	9d	9e	9f

CHO-1	9.65, s	9.66, d, $^1J_{\text{C,H}} = 185.0$	9.65, s	9.66, s	9.67, s	9.65, s	9.66, s
H-3	7.27, d, $J_{\text{H,H}} = 3.7$	7.27, d	7.27, dd, $^2J_{\text{C2,H3}} = 9.0$	7.28, dd, $^1J_{\text{C3,H3}} = 182.5$	7.28, „t“, $^2J_{\text{C4,H3}} = 3.5$	7.27, dd, $^{3,4}J_{\text{C5,H3}} = 7.3$	7.27, dd, $^4J_{\text{C6,H3}} = 0.4$
H-4	7.47, d, $J_{\text{H,H}} = 3.7$	7.47, d	7.47, dd, $^{3,4}J_{\text{C2,H4}} = 7.5$	7.49, m, $^2J_{\text{C3,H4}} = 3.0$	7.48, dd, $^1J_{\text{C4,H4}} = 181.4$	7.48, dd, $^2J_{\text{C5,H4}} = 9.5$	7.47, dd, $^3J_{\text{C6,H4}} = 5.2$

at C-1 with its axial oxycarbonyl substituent gave exclusively the α -configured methyl glycoside **6** by a second ring conversion, so that the desired product stereochemistry at C-1 was already set in this step. The synthetic “detour” via lactone **5** cannot be avoided since direct glycosidation of uronic acid donors gives inferior yields in most cases due to their low reactivity. Acid **6** was the first intermediate in the sequence that required chromatographic purification.

Selective elimination of the C-4-substituent in compound **6** was synthetically challenging. Although it is favored over the elimination of other substituents because the formed double bond is in conjugation with the carboxyl group (β -elimination), this effect is not very pronounced and—in general—elimination has to proceed from the disfavored synclinal conformation. It was known from previous work that a similar reaction starting from galacto-configured precursors went much more smoothly due to their favorable antiperiplanar arrangement (Tot et al. 2008). However, D-galactose (or even D-galactopyranosiduronic acid) is not available in suitably ^{13}C -labeled forms, so the synthesis had to cope with the respective commercially available gluco-compounds. Generally, elimination from the free acid **6** did not work better than giving a 40% yield after optimization, a rather meager outcome in view of the restraints by the costly isotopically labeled compounds. Analysis of the byproducts showed the carboxylic moiety to be centrally involved, so that protection of that function appeared to be a good idea. Indeed, protection of the COOH group as its methyl ester **7** immediately doubled the yield to about 80%, which was an acceptable outcome, especially since ester formation before the elimination and deprotection after elimination (removal of acetates from the hydroxyl groups and methyl deprotection of the carboxylic acid function) were virtually quantitative and did not further diminish the yields. The methylation of **6** was carried out with trimethylsilyl-diazomethane (Hoai et al. 2004; Tot et al. 2009). Simultaneous deprotection of COOH and OH functions in **8** was accomplished with LiOH in water/THF, (Adorjan et al. 2006) which was superior to both other alkali hydroxides and Zemplén deprotection. The elimination step itself was catalyzed by DBU in acetic anhydride/pyridine (Otonani and Yosizawa 1987).

Table 4 ^{13}C NMR (100 MHz, DMSO- d_6) data of 5-formyl-2-furancarboxylic acid (**9**) and its ^{13}C -labeled isotopomers (**9a–9f**) including chemical shifts (data in ppm) and the homonuclear (C,C) couplings (data in Hz)

	9	9a	9b	9c	9d	9e	9f

CHO-1	180.0	180.0*	180.1, d, $^1J_{\text{C,C}} = 91.0$	180.0, d, $^2J_{\text{C,C}} = 0.6$	180.0, d, $^{3,4}J_{\text{C,C}} = 2.0$	180.0, d, $^{3,4}J_{\text{C,C}} = 2.1$	180.0, d, $^4J_{\text{C,C}} = 0.3$
C-2	148.6	148.7, d, $^1J_{\text{C,C}} = 91.0$	148.6*	148.7, d, $^1J_{\text{C,C}} = 68.3$	148.6, d, $^{2,3}J_{\text{C,C}} = 2.4$	148.6, d, $^{2,3}J_{\text{C,C}} = 2.7$	148.6, d, $^{3,4}J_{\text{C,C}} = 1.0$
C-3	118.8	118.8, d, $^2J_{\text{C,C}} = 0.6$	118.9, d, $^1J_{\text{C,C}} = 68.3$	118.8*	118.8, d, $^1J_{\text{C,C}} = 49.2$	118.8, d, $^{2,3}J_{\text{C,C}} = 1.6$	118.9, d, $^{3,4}J_{\text{C,C}} = 0.8$
C-4	122.4	126.5, d, $^3J_{\text{C,C}} = 2.0$	122.4, d, $^{2,3}J_{\text{C,C}} = 2.4$	122.5, d, $^1J_{\text{C,C}} = 49.2$	122.5*, $^1J_{\text{C,C}} = 66.0$	122.5, d, $^1J_{\text{C,C}} = 66.0$	122.6, d, $^2J_{\text{C,C}} = 0.8$
C-5	153.6	153.5, d, $^3J_{\text{C,C}} = 2.1$	153.7, d, $^{2,3}J_{\text{C,C}} = 2.7$	153.6, d, $^{2,3}J_{\text{C,C}} = 1.6$	153.7, d, $^1J_{\text{C,C}} = 66.0$	153.6*, $^1J_{\text{C,C}} = 67.8$	153.7, d, $^1J_{\text{C,C}} = 67.8$
COOH-6	159.4	159.3, d, $^4J_{\text{C,C}} = 0.3$	159.2, d, $^{3,4}J_{\text{C,C}} = 1.0$	159.5, d, $^{3,4}J_{\text{C,C}} = 0.8$	159.3, d, $^2J_{\text{C,C}} = 0.8$	159.7, d, $^1J_{\text{C,C}} = 67.8$	159.5*, $^1J_{\text{C,C}} = 67.8$

The heteronuclear coupling constants (C,H) are listed in Table 3

* >99.9% ^{13}C

Table 5 ^{13}C NMR resonances of 5-formyl-2-furancarboxylic acid (**9**) recorded in D_2O and DMSO-d_6 and the heteronuclear couplings ($J_{\text{C-H}}$, data in Hz), cf. Table 3

	D_2O	DMSO-d_6	Multiplicity and $J_{\text{C-H}}$ (DMSO-d_6)

C-2	151.9	148.64	dd, $^2J = 9.0$, $^{3,4}J = 7.5$
C-3	121.6	118.80	dd, $^1J = 182.5$, $^2J = 3.0$
C-4	126.5	122.43	dd, $^1J = 181.4$, $^2J = 3.5$
C-5	155.8	153.64	ddd, $^3J = 32.3$ (OH), $^2J = 9.5$, $^{3,4}J = 7.3$
COOH-6	164.6	159.40	s
CHO-1	184.5	180.05	d, $^1J = 185.0$

The long reaction times used (72 h) are necessary for complete conversion, since reaction temperatures above 30°C must be avoided as they caused the yield to decrease drastically, going down to 58% and to 24% at 50°C and 65°C , respectively. The sequence from lactone **6** to target **1** was done as one-pot conversion with recrystallization as the final purification step. The whole sequence thus required only one intermediate column chromatography and one recrystallization step. The overall yield ranged between 42 and 46% over 7 steps, which was well acceptable also for the labeled compounds.

^{13}C isotopomers of the HexA model compound and NMR connectivities

The synthesis sequence of Scheme 2 was repeated six times, starting with D-glucuronic acids each carrying a ^{13}C -monolabel at one of the six carbon atoms. This way, the isotopically labeled HexA model compounds methyl 1- ^{13}C -4-deoxy- β -L-*threo*-hex-4-enopyranosiduronic acid (**1a**), methyl 2- ^{13}C -4-deoxy- β -L-*threo*-hex-4-enopyranosiduronic acid (**1b**), methyl 3- ^{13}C -4-deoxy- β -L-*threo*-hex-4-enopyranosiduronic acid (**1c**), methyl 4- ^{13}C -4-deoxy- β -L-*threo*-hex-4-enopyranosiduronic acid (**1d**), methyl 5- ^{13}C -4-deoxy- β -L-*threo*-hex-4-enopyranosiduronic acid (**1e**), and methyl 6- ^{13}C -4-deoxy- β -L-*threo*-hex-4-enopyranosiduronic acid (**1f**) were provided. Their structures are given in Scheme 3, indicating the ^{13}C -label by a red dot.

Before starting mechanistic studies on HexA degradation and chromophore formation, there was a more immediate benefit from the availability of the six isotopomers: a full description of the NMR spin system of methyl 4-deoxy- β -L-*threo*-hex-4-enopyranosiduronic acid. Besides the NMR chemical shifts in the ^1H and ^{13}C domain as obvious and readily available data, a complete set of connectivities became accessible, describing all coupling constants of the system, i.e. direct, vicinal, geminal and long-range coupling constants for both homonuclear (H–H and C–C) and heteronuclear (C–H) couplings. Such full connectivity data are rather rare for reasonably large spin systems (with more than 3–4 carbons) as they usually require isotopically labeled or per-labeled compounds (Kalinowski et al. 1984).

The 3J homonuclear H,H coupling was visible for all vicinal proton pairs, with values of 2.8 Hz for $^3J_{\text{H1,H2}}$ and $^3J_{\text{H3,H4}}$ and 7.6 Hz for $^3J_{\text{H2,H3}}$. The homonuclear, direct C,C couplings ranged between about 40 Hz for $^1J_{\text{C1,C2}}$, $^1J_{\text{C2,C3}}$ and $^1J_{\text{C3,C4}}$ over 54.2 Hz for $^1J_{\text{C4,C5}}$ to $^1J_{\text{C5,C6}} = 74.0$ Hz, and fell thus in the expected regions. $^2J_{\text{C,C}}$ couplings were about 2.5–3.0 Hz between C1–C3, C2–C4, C3–C5 and C5–C1 and 0.4 Hz for the C4–C6 coupling. $^3J_{\text{C,C}}$ couplings were rather small (<0.5 Hz) for C1–C4, C2–C5, and C2–(OCH₃), but larger for the couplings involving C6 (3.5 Hz to C1 via the ring oxygen and 3.6 Hz to C3). These $^3J_{\text{C,C}}$ couplings are dependent on the dihedral angle (Severson and Maciel 1984; Karplus 1963) in a way vicinal protons are, for which this dependence is usually displayed as the well-

Table 6 ^1H NMR (400 MHz, DMSO-d_6) data of 2-furancarboxylic acid (**10**) and its ^{13}C -labeled isotopomers (**10b–10f**) including the chemical shifts (data in ppm) and the homonuclear (H,H) and heteronuclear (C,H) coupling constants (data in Hz)

	10	10b	10c	10d	10e	10f

H-2	7.85, dd, $^3J = 1.8$, $^4J = 0.8$	7.86, ddd, $^1J_{\text{C2,H2}} = 205.4$	7.85, ddd, $^2J_{\text{C3,H2}} = 13.2$	7.85, ddd, $^{3,4}J_{\text{C4,H2}} = 3.7$	7.86, ddd, $^{3,4}J_{\text{C5,H2}} = 7.3$	7.85, dd
H-3	6.59, dd, $^3J = 3.5$, $^3J = 1.8$	6.60, ddd, $^2J_{\text{C2,H3}} = 11.0$	6.60, ddd, $^1J_{\text{C3,H3}} = 178.0$	6.59, ddd, $^2J_{\text{C4,H3}} = 5.5$	6.59, ddd, $^{3,4}J_{\text{C5,H3}} = 7.3$	6.59, ddd, $^4J_{\text{C6,H3}} = 0.2$
H-4	7.18, dd, $^3J = 3.5$, $^4J = 0.8$	7.18, ddd, $^{3,4}J_{\text{C2,H4}} = 7.4$	7.18, ddd, $^2J_{\text{C3,H4}} = 4.8$	7.18, ddd, $^1J_{\text{C4,H4}} = 178.7$	7.18, ddd, $^2J_{\text{C5,H4}} = 9.6$	7.18, ddd, $^3J_{\text{C6,H4}} = 1.2$

Table 7 ^{13}C NMR (100 MHz, DMSO-d_6) data of 2-furancarboxylic acid (**10**) and its ^{13}C -labeled isotopomers (**10b–10f**) including the chemical shifts (data in ppm) and the homonuclear (C,C) couplings (data in Hz)

	10	10b	10c	10d	10e	10f

C-2	145.2	145.2*	145.3, d, $^1J_{\text{C,C}} = 67.6$	145.3, d, $^{2,3}J_{\text{C,C}} = 2.1$	145.2, d, $^{2,3}J_{\text{C,C}} = 2.7$	145.2, d, $^{3,4}J_{\text{C,C}} = 2.0$
C-3	117.9	118.0, d, $^1J_{\text{C,C}} = 67.63$	117.9*	117.9, d, $^1J_{\text{C,C}} = 49.0$	117.9, d, $^{2,3}J_{\text{C,C}} = 1.5$	117.9, d, $^{3,4}J_{\text{C,C}} = 1.5$
C-4	112.3	112.3, d, $^{2,3}J_{\text{C,C}} = 2.1$	112.3, d, $^1J_{\text{C,C}} = 49.0$	112.2*	112.3, d, $^1J_{\text{C,C}} = 71.5$	112.3, d, $^2J_{\text{C,C}} = 0.8$
C-5	147.2	147.2, d, $^{2,3}J_{\text{C,C}} = 2.7$	147.2, d, $^{2,3}J_{\text{C,C}} = 1.5$	147.2, d, $^1J_{\text{C,C}} = 71.5$	147.2*	147.2, d, $^1J_{\text{C,C}} = 96.2$
COOH-6	159.6	159.9, d, $^{3,4}J_{\text{C,C}} = 2.0$	160.1, d, $^{3,4}J_{\text{C,C}} = 1.5$	159.6, d, $^2J_{\text{C,C}} = 0.8$	159.2, d, $^1J_{\text{C,C}} = 96.2$	159.5*

The heteronuclear coupling constants (C,H) are listed in Table 6

* > 99.9% ^{13}C

Table 8 ^{13}C NMR resonances of 2-furancarboxylic acid (**10**) recorded in D_2O and DMSO-d_6 and heteronuclear couplings ($J_{\text{C-H}}$, data in Hz), cf. Table 6

	D_2O	DMSO-d_6	Multiplicity and $J_{\text{C-H}}$ (DMSO-d_6)
C-2		150.32	147.17	ddd, $^1J = 205.4$, $^2J = 11.0$, $^{3,4}J = 7.4$
C-3		115.07	112.28	ddd, $^1J = 178.0$, $^2J = 13.2$, $^3J = 4.8$
C-4		122.00	117.92	ddd, $^1J = 178.8$, $^2J = 5.5$, $^{3,4}J = 3.7$
C-5		146.89	145.23	ddd, $^2J = 9.6$, $^{3,4}J = 7.3$, $^{3,4}J = 7.3$
C-6		164.91	159.61	s

Scheme 3 Structures of the isotopically labeled HexA model compounds **1a–1f**. The position of the ^{13}C -label ($>99\%$ ^{13}C) is indicated by a red dot

known Karplus curve (Karplus 1963; Marquez et al. 2001).

The direct heteronuclear couplings, i.e. $^1J_{\text{C-H}}$, were, of course, almost two orders of magnitude larger than those over two or three bonds. Longer couplings were not observed. $^1J_{\text{C-H}}$ involving the anomeric carbon and the sp^2 -hybridized C4 are 165 and 169 Hz, respectively, while those at the sp^3 -carbons C2 and C3 are around 125 Hz. The vicinal (two bonds) and geminal (three bonds) coupling fell generally between 2 and 6 Hz, with the exceptions of $^3J_{\text{C5,H3}} < 0.1$ Hz and $^2J_{\text{C5,H4}} = 11.3$ Hz. The coupling constants (connectivities) in the spin system of **1** are summarized in Scheme 4 as well as Tables 1 and 2 (see experimental section).

Stability of the HexA model compound

The labeled model compounds were expected to be quite helpful tools in monitoring the fate of HexA

during degradation and chromophore formation by following a specific carbon through this process. Since this can be done for every single carbon in the system, a maximum of information on the reaction mechanism was anticipated. It is known that HexA is completely degraded under the conditions of an industrial hot acid treatment (so-called “A stage”). High temperatures ($T > 80$ °C) degrade HexA units in xylan or pulp as well as the HexA model compound **1** completely within less than 3 h, high acidities ($\text{pH} < 3$) having the same effect in even shorter times. At a temperature of 100 °C and a pH of 2, compound **1** was completely destroyed in 50 s, at 80 °C and pH 3 in 120 s. At first a brownish solution is formed which turns almost black after 1 h and forms a black precipitate upon standing at room temperature overnight (see Fig. 1). The question of the nature of these intensely colored compounds will be addressed in the following part of this series on chromophores. Under milder conditions (e.g. $\text{pH} = 4.5$, 50 °C), model compound **1** is still consumed (albeit much more slowly in the order of

Scheme 4 Full analysis of the NMR connectivities (homonuclear and heteronuclear couplings) in HexA model compound **1**. Couplings are displayed with arrows; J values are given in Hz throughout

10–20 h), but no visible color is generated. This color formation commences as soon as the temperature is increased or the pH value decreased.

Primary degradation products of HexA: their stability, color and interrelation

It is known from literature that degradation of HexA can generate furan compounds, such as 5-formylfuran-2-carboxylic acid (**9**) (Clavijo et al. 2012; Teleman et al. 1996). In this study, we were able to demonstrate that the three compounds 5-formylfuran-2-carboxylic acid (5-formyl-2-furoic acid, **9**), furan-2-carboxylic acid (2-furoic acid, **10**) and formic acid (**11**) were isolable intermediates of the HexA degradation. In addition, they are the *only* three compounds formed as

initial degradation intermediates and they are obtained quantitatively under appropriately mild conditions: their masses add up to 100% of the starting material. In most previous studies these compounds had not been detected quantitatively as the degradation conditions were too severe so that these intermediates were almost immediately consumed further. Treatment of **1** at 50 °C in weakly acidic medium (pH = 4.5) generates a mixture of **9**, **10**, and **11** after 19 h (see Fig. 1 and experimental section). Note that these conditions are different from just dissolving **1** in distilled water. A 10 mM solution of **1** in distilled water (non-buffered) produces an immediate pH of 4.9 (note that **1** is a carboxylic acid) and the pH drops further due to degradation to a final pH of 3.6 after 5 h at 50 °C. All three initial degradation products are

Fig. 1 Degradation of HexA model compound **1** under mild conditions (0.1 M, 50 °C, pH 4.5) into a colorless mixture of 5-formyl-2-furoic acid (**9**), furan-2-carboxylic acid (2-furoic acid, **10**) and formic acid (**11**). Under harsher conditions, a *brownish* solution is furnished that quickly turns *black* and produces a black precipitate upon longer standing. (Color figure online)

organic acids and are responsible for the drop in pH upon degradation of **1**. At 50 °C and higher acidity (pH 3, phosphate buffer), consumption of **1** and degradation into **9**, **10**, and **11** was much faster and already complete after 23 min.

It should be noted that the three initial degradation intermediates are not chromophores *per se* (as erroneously stated in many literature accounts). The same applies, by the way, also to similar furan compounds, such as furfural or hydroxymethylfurfural (HMF). That formic acid (**11**) is colorless is commonly known, but also 2-furoic acid (**10**) in pure form is a white solid (m.p. $\sim 130^\circ\text{C}$), as is 5-formyl-2-furoic acid (**9**, m.p. $\sim 211^\circ\text{C}$). However, both **9** and **10** are quite unstable towards oxygen (ambient air), acids, strong bases and heat, and so they rarely occur in pure form. If not freshly purified, they are accompanied by strongly colored degradation products, so that their appearance is usually yellowish to brownish. When kept standing at room temperature in air at a relative humidity of 60%, both compounds generate byproducts, which is reflected by a constantly decreasing melting point, so that after some days an oil is obtained. An atmosphere containing some acetic acid vapor (0.1 ml of glacial

acetic acid in a gas volume of 2 l) degrades both compounds completely to brownish oil within 8 h, while hydrogen chloride vapor (0.1 ml of 1 M aqueous HCl in a gas volume of 2 l) causes formation of a black tar already within 20 min.

It is thus certainly true that both 5-formyl-2-furoic acid (**9**) and 2-furoic acid (**10**) are potent *chromogens* (from Ancient Greek: $\chi\rho\omega\mu\alpha$ = color and $\gamma\epsilon\gamma\epsilon\sigma\omega$ = origin, formation), i.e. they are able to produce chromophores, they are chromophore *precursors*. But neither of them is a *chromophore* itself (from Ancient Greek: $\chi\rho\omega\mu\alpha$ = color and $\phi\epsilon\rho\epsilon\iota\nu$ = to carry). Formic acid (**11**), as the third HexA degradation product, is evidently neither a chromogen nor a chromophore.

Interestingly, when HexA model compound **1** was stressed under mild degradation conditions (50 °C, pH 4–5), always the same molar amounts of 2-furoic acid (**10**) and formic acid (**11**) were formed, i.e., their ratio was always 1. Moreover, the sum of 5-formyl-2-furoic acid (**9**) and 2-furoic acid (**10**) was constant, with **9** being slowly consumed and **10** being formed at the same rate. It was thus logical to assume that **9** was the precursor of both **10** and **11**. Indeed, under the conditions of mild degradation of HexA model

Fig. 2 ^1H NMR spectra of the slow conversion of 5-formyl-2-furoic acid (**9**, abbrev. FFA, top spectrum) into 2-furoic acid (**10**, abbrev. FCA) and formic acid (**11**, abbrev. FA) at pH 4. The reaction mixture was analyzed after different reaction times (approx. every 20 min, middle spectra 2–5) with the approximate molar ratios given above the trace. The molar ratio of **10** and **11** was constant at 1:1 throughout, and the two compounds were the only products after complete consumption of **9** (bottom spectrum)

compound **1**, 5-formyl-2-furoic acid (**9**) is slowly degraded into an equimolar mixture of 2-furoic acid (**10**) and formic acid (**11**), see Fig. 2. At a pH of 4.5, the rate of the degradation of **9** is somewhat slower than that of **1**, but in the same order of magnitude, so that shortly after consumption of **1** mixtures of **9**, **10** and **11** are obtained (see experimental section), but at longer reaction times only **10** and **11** are left. At pH values below 3, both furoic acids **9** and **10** are decomposed so quickly that they cannot be isolated in pure form.

At 50 °C and at a pH of 5.5–7, only **9** is formed in initial phases of the decomposition reaction but not yet **10** (and **11**). Even after several days only very small amounts of **10** and **11** are formed, while **1** had already been fully consumed. At a pH of 4–5.5, consumption of **1** with production of **9** is accelerated, but at the same time also the further conversion to **10** and **11**, so that **10** and **11** become more and more dominating with prolonged reaction times (*cf.* experimental section and Fig. 2). At a pH between 3.0 and 4.0 degradation of **1** is quite fast on the order of less than an hour, and **9** becomes a short-lived intermediate, whose stationary concentration is rather low: a few minutes after complete consumption of **1**, only **10** and **11** (but no more **9**) are found. As mentioned above, both **9** and **10** are very rapidly degraded below a pH of 3 and cannot be detected in such acidic media.

A detailed kinetic description (rate constants and Arrhenius activation energies) of the reaction system $\mathbf{1} \rightarrow \mathbf{9} \rightarrow \mathbf{10} + \mathbf{11}$ has not yet been performed since it did not seem crucial with regard to the main aim of chromophore identification and formation mechanisms. It was already evident, however, that the rate of the degradation of **1** was dependent on both the pH value (*i.e.* the proton concentration) and the model compound's concentration. The same was true for the consumption of **9**. Therefore second-order kinetics appeared likely for both degradation processes, thus giving a system of coupled follow-up reactions with **9** playing the role of an intermediate whose stability (and thus stationary concentration) decreased with increasing acidity of the medium.

It is understood that the mild conditions are not relevant for industrial bleaching that is done at much more drastic settings, but they are necessary for mechanistic studies in order to get hold of the unstable furoic acid intermediates **9** and **10** (and the stable product **11**).

Mechanism of formation of the primary HexA degradation products

The mechanism of formation of 5-formyl-2-furoic acid (**9**) from HexA units is readily understood (see Scheme 5). A furan moiety cannot be formed unless

Scheme 5 Mechanism of formation of 5-formyl-2-furoic acid (**9**) from HexA model compound methyl 4-deoxy-β-L-threo-hex-4-enopyranosiduronic acid (**1**). Intermediates **I-1** and **I-2** were detectable by NMR spectroscopy with the help of

isotopically labeled starting material despite their low concentration, whereas intermediates **I-3** and **I-4** were too short-lived for such direct monitoring, even with ¹³C-labeled compounds

the six-membered ring in **1** is opened. This requires acidic media as **1** is quite stable in neutral and moderately alkaline medium (the degradation in strong alkali follows a different mechanism which cannot be discussed here). The first elementary step in the sequence is thus protonation of the ring oxygen as the most nucleophilic position with concomitant ring opening. This seems to be the rate determining step, which also explains the involvement of H⁺ in the rate law (see above). The ring opening itself corresponds to the cleavage of the enol ether structure, for which generally two mechanisms are discussed that are distinguished by the order of the two elemental steps: either initial cleavage of the ether (the C1–O5 bond) followed by tautomerization of the enol to the ketone, or initial [1, 3]-sigmatropic proton shift (from the protonated ring oxygen to C4) with concomitant ether bond (C1–O5) cleavage. At the same time water is added to the anomeric carbon with loss of methanol from the resulting hemiacetal, producing the α-ketoacid intermediate **I-1** (Scheme 5). In an DMSO-d₆/D₂O mixture (v/v = 3:1), the open-chain form of **I-1** can be detected, having a ¹³C resonance at 205 ppm, albeit only in the case of the 5-¹³C-labeled starting material **1e** with its “enforced” C5-sensitivity. In ¹³C NMR experiments, the sensitivity of the labeled positions (>99% ¹³C) is increased by a factor of about

100 in comparison to the non-labeled compound with natural ¹³C abundances throughout (~1%).

Hypothetical protonation at the glycosidic oxygen appears to play no role in the mechanism: in that case competitive nucleophiles present (fluoride, cyanide, diethyl malonate) should trap the intermediate C1-cation by retention of the pyranose ring, which was not observed. Instead, all trapping products were ring-opened, having an α-keto structure similar to **I-1**.

The next step is the entropically driven formation of a furan ring **I-2** by nucleophilic attack of the C2-hydroxyl at the 5-keto carbon. By means of the ¹³C-labeled compounds, also this intermediate can be detected despite its very low concentration; characteristic resonances are C1 at 181.3 ppm (seen when starting from C1-labeled compound **1a**) and C5 at 95.4 ppm (seen when starting from C5-labeled compound **1e**) in DMSO-d₆/D₂O (v/v = 3:1). From this intermediate, two eliminations of water, establishing the double bonds between C2–C3 and C4–C5, are required to form furan derivative **9**. These eliminations appeared to be very fast, and neither of the hypothetical intermediates **I-3** and **I-4** could be detected, not even at lower temperatures. When acidity and temperature were sufficient to induce ring opening of **1** and thus formation of the transient intermediates **I-1**/**I-2**, the subsequent processes leading to **9** were

Scheme 6 Proposed mechanism of the loss of formic acid (**11**) from 5-formyl-2-furoic acid (**9**) under formation of 2-furoic acid (**10**). Note that—while the aldehyde hydrate **I-5** is detectable in

special solvents and the individual steps are in line with organic chemistry rules—the mechanism fails to explain why **9** undergoes deformylation, but other furfural derivatives do not

apparently so much favored that they occurred “automatically”.

A possible mechanism for the further conversion of 5-formyl-2-furoic acid (**9**) into 2-furoic acid (**10**) and formic acid (**11**) is shown in Scheme 6. The reaction is formally a deformylation (in general: deacylation) of the 2-furoic moiety, which usually does not occur; strongly acidic media would catalyze the reaction in principle, but this would rather cause degradation of the furan system than deacylation. A hint as to a possible mechanism was the fact that in strongly polar NMR solvents, such as DMAc/LiCl (Adelwoehrer et al. 2008) or ionic liquids, such as BMIM acetate (Yoneda et al. 2009), the formyl group in **9** was not only present as aldehyde (–CHO), but also in the form of an aldehyde hydrate (–CH(OH)₂), if the solvent contained some water. In perdeuterated BMIM acetate containing 5% of D₂O, the ratio between aldehyde and aldehyde hydrate at 20 °C was 87:13, estimated from the ¹³C resonances at 186.3 ppm and 98.8 ppm, respectively. Assuming that the hydrate form would also be present in weakly acidic water medium to small extent (it was not detectable by NMR, however), a deformylation under temporary acid-catalyzed dearomatization of the furan followed by subsequent rearomatization is mechanistically plausible (see Scheme 6).

The furan system’s weak aromaticity can be canceled relatively easily (Achmatowicz et al. 1971), so a dearomatization/rearomatization sequence would *per se* be conceivable. The fact that deformylation did not occur upon thermal treatment in water at neutral pH, but required some weak acidity, might be seen as support for that mechanism. However, it cannot be

answered why 5-formyl-2-furoic acid (**9**) undergoes deformylation, but furfural and 5-hydroxymethylfurfural (HMF) do not, as is experimentally confirmed. It would be remarkable that the COOH group in **9** changes the reactivity of the furan moiety so profoundly and selectively that deformylation occurs, while it does not in the case of other substituents (–H in furfural or –CH₂OH in HMF). At present, only the neat deformylation of **9** in weakly acidic aqueous medium can be stated, but we are unable to offer a convincing mechanistic rationale for this process.

In context with the mechanistic considerations it should eventually be mentioned that reductic acid (2,3-dihydroxy-cyclopent-2-en-1-one) was not found as a primary degradation intermediate of HexA and only in negligibly small concentrations in later stages of HexA degradation (see next part of this series on the structure of the HexA-derived chromophores). The compound has been proposed to be an intermediate in the color formation of HexA (Sevastyanova et al. 2006a, b). We do not claim that reductic acid plays no role in the degradation chemistry of HexA, we were just unable to confirm its presence both in our model experiments under mild conditions and in HexA degradation experiments under more harsh conditions resembling industrial “A stages” or bleaching sequences.

¹³C isotopomers of the primary degradation products and their NMR connectivities

The degradation of methyl 4-deoxy-β-L-*threo*-hex-4-enopyranosiduronic acid (**1**) into 5-formyl-2-furoic acid (**9**) and further to 2-furoic acid (**10**), see

Scheme 7 Structures of the ^{13}C -isotopomers of 5-formyl-2-furoic acid (**9a–9f**) and of 2-furoic acid (**10b–10f**) derived from the isotopically labeled HexA model compounds **1a–1f**. Degradation of **9a** provides non-labeled **10** and ^{13}C -formic acid

Scheme 6, was repeated six times, starting with the different ^{13}C -isotopomers **1a–1f**, which each had a ^{13}C -monolabel at one of the six carbon atoms. This way, six different isotopomers of 5-formyl-2-furoic acid (**9**) and five isotopomers of 2-furoic acid (**10**) were obtained. In the case of 5-(^{13}C -formyl)-2-furoic acid (**9a**) the isotopic marker is “lost” in the form of ^{13}C -formic acid (see experimental section). The structure of the compounds is given in Scheme 7, the position of the ^{13}C -label being indicated again by a red dot.

Also for the furan compounds **9** and **10** a full description of the NMR spin systems was possible by means of the isotopically labeled specimens, including the chemical shifts and all homonuclear and heteronuclear couplings. The 3J homonuclear H3–H4 coupling was quite similar, around 3.5 Hz, in both systems, the 4J coupling in **10** (H2–H4) being 0.8 Hz. The direct homonuclear C,C couplings within the furan system ranged between 49 and 72 Hz, the coupling between C3 and C4 (~ 49 Hz) being smaller in both compounds by about 20 Hz than the other direct couplings in the ring system (~ 70 Hz). Interestingly, the $^1J_{\text{C,C}}$ coupling to the carbonyl carbons, which is usually about 90–100 Hz, was 91 Hz and 96.2 for the carboxyl carbons in **9** and **10**, respectively, but only 67.8 Hz for the CHO group in **9**. Whether this correlates with a special chemical behavior as indicated in Scheme 6 cannot be answered at the moment.

$^2J_{\text{C,C}}$ couplings were about 0.8 Hz throughout both furan systems and also to the exocyclic carbon substituents, and just significantly smaller than in the spin system of **1** (2.5–3.0 Hz in the ring). Interestingly, due to the five-membered ring structure, there are no “pure” $^3J_{\text{C,C}}$ couplings in the spin systems, since they are always superimposed by another coupling over a different bond number, either as $^{2,3}J_{\text{C,C}}$ or as $^{3,4}J_{\text{C,C}}$. In simpler words, the coupling path between the furans’ carbons can be “clockwise” or “counter-clockwise”, and both contributions mix to the final value. The coupling of C2 with C4, for instance, is a 2J coupling via C3, and a 3J coupling via O and C5. All $^{2,3}J_{\text{C,C}}$ and $^{3,4}J_{\text{C,C}}$ values are in the range of 2 Hz, with the exception of the couplings to the COOH group in **9** (0.8 and 1 Hz, respectively). The only present $^4J_{\text{C,C}}$ —which would actually be a $^{4,5}J_{\text{C,C}}$ value—is 0.3 Hz, occurring between CHO and COOH in compound **9**.

The direct heteronuclear couplings, i.e. $^1J_{\text{C-H}}$, were all about 180 Hz, both for the aromatic protons in the furan system and in the CHO, with the exception of C2 in **10** with the rather high coupling of 205 Hz. All of these carbons are sp^2 -hybrids. All longer-range couplings, i.e. $^2J_{\text{C,H}}$ and $^3J_{\text{C,H}}$, are in the expected range between 5 and 15 Hz. Interestingly, while the carboxylic carbons in both **9** and **10** show a heteronuclear coupling with the furan protons, the CHO carbon in **9** does not, nor does the aldehyde proton connect to the ring carbons—a possible correlation of these NMR

peculiarities with the special deformylation behavior once more being unknown.

Conclusions

Yellowing, bleaching and brightness reversion are evidently “big topics” in pulp and paper manufacturing, science of cellulosic materials in general, and conservation science, and it is also obvious that hexeneuronic acid is a potent contributor to bleaching, brightness and brightness reversion phenomena. At the same time, the topic is only relevant for wood starting materials and their mixtures, which contain D-glucuronoxylan, of which the 4-*O*-methylglucuronic acid residues are converted to HexA moieties during pulping. Still, hardwood pulps are frequently used, making the topic of HexA-derived chromophores not only scientifically challenging, but also economically highly important. From the studies applying the CRI method to isolate chromophores from cellulosic matrices in order to determine their structures we have learned that the concentration of individual chromophores is extremely low: in the low ppm to ppb range. Both the unambiguous confirmation of the chromophores’ structures as well as kinetic or mechanistic studies required the synthesis of authentic samples. To link those structures with possible precursors—such as model compounds of oxidized anhydroglucose units in cellulose—was only possible by means of isotopic labeling with ^{13}C -markers. Otherwise, the complexity of mechanisms and product mixtures overwhelm the analytical capabilities. With the instrument of labeling, the fate of particular carbons can be tracked along a process, and fragmentations or condensations/recombinations can be conveniently followed. It was logical to apply the same tool for similar mechanistic studies concerning HexA.

The synthesis of the HexA moiety (with a truncated xylan backbone mimicked by a methyl group) was straightforward, but required careful optimization of each step to boost yield and byproduct recovery to lose as little of the costly ^{13}C -labeled starting material as possible. An approx. 45% overall yield over 7 steps was satisfying. The same sequence provided six ^{13}C -isotopomers with labels at each of the respective six carbons (non-labeled compound **1** and isotopomers **1a–1f**).

The HexA model compound is highly unstable at elevated temperatures ($T > 50\text{ }^{\circ}\text{C}$) and in acidic media

($\text{pH} < 6$). Removal of HexA residues in bleaching sequences is done in so-called “A stages”, which work with acid at elevated temperatures (Chakar et al. 2000; Johansson and Samuelson 1977; Clavijo et al. 2012). Under those conditions, dark colored chromophores are very rapidly formed (seconds to few minutes) from HexA, and these processes are too fast for any detailed mechanistic or kinetic studies. Fortunately, reduced acidity and moderate temperatures ($\text{pH} \sim 4.5$, $T = 50\text{ }^{\circ}\text{C}$) slowed down HexA degradation in a way that chromophore formation was slowed down and even primary degradation intermediates could be captured. Under suitably mild conditions, HexA (**1**) degrades neatly into 2-formyl-furan-5-carboxylic acid (**9**), which—in a slightly slower process—is further degraded to furan-2-carboxylic acid (**10**) and formic acid (**11**). Kinetically, formation and consumption of **9** is described by a system of two consecutive reactions; conditions must be carefully controlled if higher amounts of **9** are to be isolated. At longer reaction times **9** will be consumed and only **10** is found (besides accompanying formic acid). The mechanism of the formation of **9** from HexA includes ring opening to an open-chain α -ketoacid intermediate, which in turn undergoes ring closure to a furanose derivative. Both intermediates can be directly observed by NMR. Further double dehydration forms the furan product **9**. Its subsequent conversion into **10** can be explained by a formally correct deformylation mechanism. However, it remains an open question why **10** would (relatively easily) undergo deformylation, but structurally quite similar compounds such as furfural or hydroxymethyl-furfural do not. The neat conversion of **1** into a mixture of **9** and **10** allowed for the synthesis of these compounds also as respective ^{13}C -isotopomers (**9a–9f** and **10b–10f**), starting from the respective labeled compounds **1a–1f**.

With all these compounds at hand, it was now possible to address the topic of what the chemical structures of the HexA-derived chromophores would be and how they are formed. This will be the topic of the following part of this series.

Acknowledgments Open access funding provided by University of Natural Resources and Life Sciences Vienna (BOKU). The authors would like to acknowledge the financial support of the Austrian Christian Doppler Research Society (Christian-Doppler laboratory “Advanced cellulose chemistry and analysis”) and the Austrian Forschungsförderungsgesellschaft (FFG) for financial support through project 847169 (“Chromophores II”).

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Achmatowicz A, Bukowski P, Szechner B, Zwierzchowska Z, Zamojski A (1971) Synthesis of methyl 2,3-dideoxy-DL-alk-2-enopyranosides from furan compounds. General approach to the total synthesis of monosaccharides. *Tetrahedron* 27:1973–1996
- Adelwoehrer C, Yoneda Y, Nakatsubo F, Rosenau T (2008) Synthesis of the perdeuterated cellulose solvents N-methylmorpholine N-oxide (NMMO-d11) and N,N-dimethylacetamide (DMAc-d9). *J Label Compd Radiopharm* 51(1):28–32
- Adorjan I, Potthast A, Rosenau T, Sixta H, Kosma P (2005) Discoloration of cellulose solutions in N-methylmorpholine-N-oxide (Lyocell). Part 1: studies on model compounds and pulps. *Cellulose* 12(1):51–57
- Adorjan I, Jääskeläinen A, Vuorinen T (2006) Synthesis and characterization of the hexenuronic acid model methyl 4-deoxy- β -L-*threo*-hex-4-enopyranosiduronic acid. *Carbohydr Res* 341:2439–2443
- Chakar FS, Allison L, Ragauskas AJ, McDonough TJ (2000) Influence of hexenuronic acids on US bleaching operations. *Tappi J* 83:62
- Clavijo L, Cabrera MN, Kuitunen S, Liukko S, Rauhalta T, Vuorinen T (2012) Changes in a eucalyptus kraft pulp during a mild acid treatment at high temperature. *Papel* 73:59–64
- Clayton DW (1963) The alkaline degradation of some hardwood 4-O-methyl-D-glucuronoxylans. *Svensk Papperstidn* 66:115–124
- Hoai PM, Tsunoi S, Ike M, Inui N, Tanaka M, Fujita M (2004) Dicarboxylic degradation products of nonylphenol polyethoxylates: synthesis and identification by gas chromatography-mass spectrometry using electron and chemical ionization modes. *J Chromatogr A* 1061(1):115–121
- Johansson MH, Samuelson O (1977) Epimerization and degradation of 2-O-(4-O-methyl- α -D-glucopyranosyluronic acid)-D-xylitol in alkaline medium. *Carbohydr Res* 54:295–299
- Kalinowski H-O, Berger S, Braun S (1984) ^{13}C -NMR-Spektroskopie. Georg Thieme Verlag Stuttgart, New York
- Karplus M (1963) Vicinal proton coupling in nuclear magnetic resonance. *J Am Chem Soc* 85(18):2870–2871
- Korntner P, Hosoya T, Dietz T, Eibinger K, Reiter H, Spitzbart M, Röder T, Borgards A, Kreiner W, Mahler AK, Winter H, French AD, Henniges U, Potthast A, Rosenau T (2015) Chromophores in lignin-free cellulosic materials belong to three compound classes. Chromophores in cellulose, XII. *Cellulose* 22(2):1053–1062
- Li J, Gellerstedt G (1997) The contribution to kappa number from hexenuronic acid groups in pulp xylan. *Carbohydr Res* 302:213–218
- Marquez BL, Gerwick WH, Williamson RT (2001) Survey of NMR experiments for the determination of $nJ(\text{C}, \text{H})$ heteronuclear coupling constants in small molecules. *Magn Reson Chem* 39(9):499–530
- Otonari N, Yosizawa Z (1987) Preparation of phenyl 4-deoxy- α - and β -L-*threo*-hex-4-enopyranosiduronic acids and determination of the anomeric specificity of the Δ -4,5-glycosiduronase induced from *Flavobacterium heparinum* with heparin and chondroitin sulfate. *Carbohydr Res* 159:25–36
- Potthast A, Rosenau T, Sartori J, Sixta H, Kosma P (2002) Hydrolytic processes and condensation reactions in the cellulose solvent system N,N-dimethylacetamide/lithium chloride. *Polymer* 44(1):7–17
- Potthast A, Rosenau T, Kosma P, Saariaho A-M, Vuorinen T (2005) On the nature of carbonyl groups in cellulosic pulps. *Cellulose* 12(1):43–50
- Potthast A, Schiehsler S, Rosenau T, Kostic M (2009) Oxidative modifications of cellulose in the periodate system—Reduction and beta-elimination reactions. *Holzforschung* 63(1):12–17
- Rosenau T, Potthast A, Milacher W, Hofinger A, Kosma P (2004) Isolation and identification of residual chromophores in cellulosic materials. *Polymer* 45(19):6437–6443
- Rosenau T, Potthast A, Milacher W, Adorjan I, Hofinger A, Kosma P (2005a) Discoloration of cellulose solutions in N-methylmorpholine-N-oxide (Lyocell). Part 2: isolation and identification of chromophores. *Cellulose* 12(2):197–208
- Rosenau T, Adorjan I, Potthast A, Kosma P (2005b) Isolation and identification of residual chromophores in cellulosic materials. *Macromol Symp* 223(1):239–252
- Rosenau T, Potthast A, Kosma P, Suess H-U, Nimmerfrohn N (2007) Isolation and identification of residual chromophores from aged bleached pulp samples. *Holz-forschung* 61(6):656–661
- Rosenau T, Potthast A, Kosma P, Suess H-U, Nimmerfrohn N (2008) Chromophores in aged hardwood pulp—their structure and degradation potential. *Tappi J* 1:24–30
- Rosenau T, Potthast A, Krainz K, Yoneda Y, Dietz T, Shields ZPI, French AD (2011) Chromophores in cellulose, VI. First isolation and identification of residual chromophores from aged cotton linters. *Cellulose* 18(6):1623–1633
- Rosenau T, Potthast A, Krainz K, Hettegger H, Henniges U, Yoneda Y, Rohrer C, French AD (2014) Chromophores in cellulose, XI: isolation and identification of residual chromophores from bacterial cellulose. *Cellulose* 21(4):2271–2283
- Sevastyanova O, Li J, Gellerstedt G (2006a) On the reaction mechanism of the thermal yellowing of bleached chemical pulps. *Nordic Pulp Pap Res J* 21:188–192
- Sevastyanova O, Li J, Gellerstedt G (2006b) Influence of various oxidizable structures on the brightness stability of fully bleached chemical pulps. *Nordic Pulp Pap Res J* 21:49–53
- Severson ML, Maciel GE (1984) A molecular orbital study of the dihedral angle dependencies of vicinal carbon-carbon coupling constants. *J Magn Reson* 57(2):248–268
- Shimizu K (1981) β -Elimination of 2-O-(4-O-methyl- α -D-glucopyranosyluronic acid)-D-xylose with methylsulfinyl carbanion and hydrolysis of the hex-4-enopyranosiduronic linkage. *Carbohydr Res* 92:219–224

- Teleman A, Harjunpaa V, Tenkanen M, Buchert J, Hausalo T, Drakenberg T, Vuorinen T (1995) Characterization of 4-deoxy- β -L-*threo*-hex-4-enopyranosyluronic acid attached to xylan in pine kraft pulp and pulping liquor by carbon-13 and proton NMR spectrometry. *Carbohydr Res* 272:55–71
- Teleman A, Hausalo T, Tenkanen M, Vuorinen T (1996) Identification of the acidic degradation products of hexenuronic acid and characterization of hexenuronic acid-substituted xylooligosaccharides by NMR spectroscopy. *Carbohydr Res* 280:197–208
- Tot I, Krainz K, Potthast A, Suess H-U, Rosenau T (2008) Comparative synthesis studies towards methyl and phenyl 4-deoxy- β -L-*threo*-hex-4-enopyranosiduronic acid as model compounds of hexenuronic acid moieties in hardwood pulps. *Macromol Symp* 262:182–189
- Tot I, Müller Y, Werner C, Rosenau T, Potthast A (2009) A novel, mild and selective methylation of carboxyl groups in cellulosic pulps. *Holzforschung* 63(6):657–664
- Yoneda Y, Ebner G, Takano T, Nakatsubo F, Potthast A, Rosenau T (2009) Synthesis of the perdeuterated cellulose solvents 1-ethyl-3-methylimidazolium acetate (EMIM-OAc- d_{14}) and 1-butyl-3-methylimidazolium acetate (BMIM-OAc- d_{18}) and of 2- ^{13}C -butyl-3-methylimidazolium acetate. *J Label Compd Radiopharm* 52(6):223–226