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NMR Isotope Tracking Reveals Cascade Steps in Carbohydrate Conversion by Sn-Beta

Samuel G. Elliot,^[a] Esben Taarning,^{[b]*} Robert Madsen,^[a] and Sebastian Meier^{[a]*}

Quantitative isotope tracking studies were used to investigate the reaction pathways occurring for Sn-Beta catalyzed carbohydrate conversion to various alpha-hydroxy esters. Experimental insight into the conversion of pentoses was sought (i) by identifying pathways based on isotope patterns in the reaction products and (ii) through probing asymmetric isotope incorporation into products. The results indicate that reaction intermediates remain coordinated to the active site throughout the reaction cascades, regardless of the reaction pathway. A predominant transformation of the C1 carbohydrate position to the C3 position of methyl lactate resembles enzymatic glycolysis. Likewise, the majority of retro-aldol cleavage occurs from the carbohydrate in the ketose form, again resembling biological glycolysis. In addition, various side-activities are detected in Sn-Beta catalyzed carbohydrate conversion, including 5,1-hydride and 1,2-carbon shift reaction of the carbohydrates.

Introduction

The study of carbohydrates as alternative resources to petrochemicals has been intensifying in recent years. Despite this large interest, many routes of carbohydrate conversion to useful chemicals are complex and lack fundamental pathway understanding. The overall complexity of carbohydrate conversion in chemocatalytic pathways is reminiscent of the complexity encountered in biochemical pathways. Analytical methods that deal with challenging metabolic reaction systems are therefore conceivable additions to the toolbox of biomass research. Recently, we have shown that high-field NMR spectroscopy enables the identification, quantification and optimization of reactants at natural isotope enrichment in biomass conversion.^[1] In addition, NMR spectroscopy may provide additional mechanistic insight into reaction cascades through the characterization of isotope distributions in the products.^[2-8] Herein, we focus on the Sn-Beta catalyzed conversion of abundant carbohydrates to methyl lactate (ML) and other alpha-hydroxy ester coproducts (Figure 1).

Production of lactate precursors for poly lactic acid production using tin-containing silicate chemocatalysis has been studied intensively since 2009.^[9] Subsequent research has

illuminated some mechanistic details of the reactions forming methyl lactate (ML) from trioses and hexoses in methanol solvent. These developments resulted in increasingly feasible methods for tuning reaction selectivity,^[1, 4, 10-14] as well as discovery of new reaction products.^[14-20] Most of these new products are alpha-hydroxy acids like lactate and some show potential as polymer building blocks.^[18, 20-23] These biomonomers include methyl 2,5,6-trihydroxy-3-hexenoate (THM), methyl 2,5-dihydroxy-3-pentenoate (DPM), methyl vinyl glycolate (MVG), assorted 3-deoxy esters (3DE) and lactones (3DL), the structures shown in Figure 1. Pathways leading to the formation of these compounds have so far remained less comprehensively studied than those of lactate formation, as these products were characterized more recently, and because the key C₄-C₆ intermediates are not as easily accessible in pure forms as analogous C₃ compounds for use as reference standards and prospective reaction substrates.

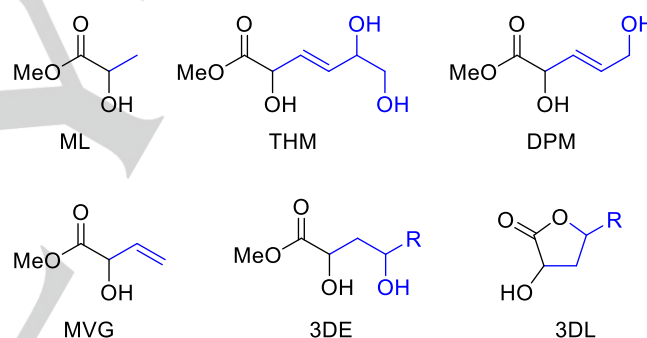


Figure 1. Product structures in the Sn-Beta catalyzed conversion of common carbohydrates, including methyl lactate (ML), methyl 2,5,6-trihydroxy-3-hexenoate (THM), methyl 2,5-dihydroxy-3-pentenoate (DPM), methyl vinyl glycolate (MVG), assorted 3-deoxy esters (3DE) and lactones (3DL). The R group of 3DE and 3DL may be either H, CH₂OH or CH(OH)CH₂OH for tetroses, pentoses or hexoses, respectively.

In the current study, we use various pentose isotopologues (pentoses with different isotope composition, i.e. different neutron content) to elucidate mechanistic details in the conversion of carbohydrates to alpha-hydroxy esters by Sn-Beta in methanol. We hypothesized that products and byproducts formed through the same pathway should be identifiable by similar isotope patterns. In addition, the formation of methyl lactate through retro-aldol cleavage of a carbohydrate to dihydroxyacetone or glyceraldehyde was reinvestigated. To this end, the symmetry of isotope incorporation was probed for products that might have arisen from symmetric dihydroxyacetone. Such experimental methods employing isotope tracking should have the prowess to identify consecutive steps in multistep-reaction pathways that occur in a concerted manner at the zeolite active sites.

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Results and Discussion

Initial experiments were conducted using D-[1-¹³C]-xylose and uniformly ²H labeled D-[UL-²H₆]-ribose isotopologues (it has previously been demonstrated that the pentose stereochemistry does not affect reaction selectivity^[1]). Pentoses were selected due to their inherently asymmetric aldol cleavage reactions that enable further distinction of product origins. Pentoses and hexoses have been found to produce an equivalent set of products^[1, 14, 19-20] and it is therefore reasonable to assume that mechanistic findings for pentoses can be transferred to the mechanisms of hexose conversion and *vice versa*.

Methyl Lactate

The mechanism of methyl lactate formation from trioses has been studied previously for both homogeneous and heterogeneous tin systems.^[3, 9, 24-26] The mechanism is proposed to consist of the following five sequential steps: a) enolization, b) dehydration, c) tautomerization, d) (hemi)acetalization and e) hydride shift (Figure 2). The starting substrate may be either a ketose or aldose, as isomerization by a 1,2-intramolecular hydride shift will result in a mixture of both forms. Previous studies were conducted using protonated substrates in deuterated solvent and enolization was deduced from deuterium incorporation into the C3 position of ML.^[3] A detailed understanding of the occurring process was sought by sensitive detection and accurate quantification of hydrogen incorporation from the solvent. In addition, we sought to warrant that intramolecular hydride shifts can be distinguished from exchange with hydrogen atoms from the heterogeneous catalyst.

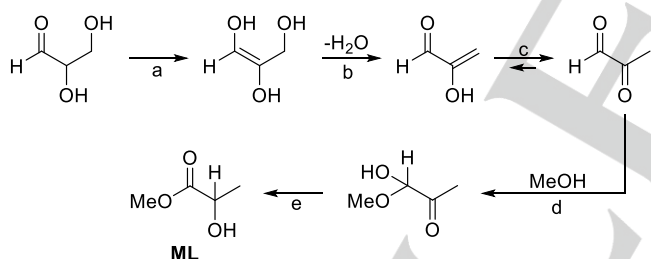


Figure 2. Plausible mechanism for the formation of lactate from trioses from the literature.^[3, 9, 24-26] Either dihydroxyacetone or glyceraldehyde may be employed as starting substrates.

We used deuterated pentose in protonated solvent to ensure that substrate and solvent as well as substrate and catalyst carried different isotopic forms of hydrogen. Resultant isotopologues of lactate were characterized by NMR spectroscopy at 18.7 Tesla magnetic field (800 MHz instrument equipped with cryogenically cooled detection electronics) using a previously demonstrated quantitative ¹³C NMR (qNMR) approach.^[1] Deuterated carbon positions were identified from characteristic chemical shift changes to lower frequency and from characteristic multiplet patterns due to coupling to quadrupolar ²H nuclei, as shown in Figure 3A. ¹³C spectra were acquired with recycle delays of 60 seconds and using inverse-gated decoupling to avoid nuclear Overhauser enhancement of ¹³C signal intensities for carbons bound to ¹H.

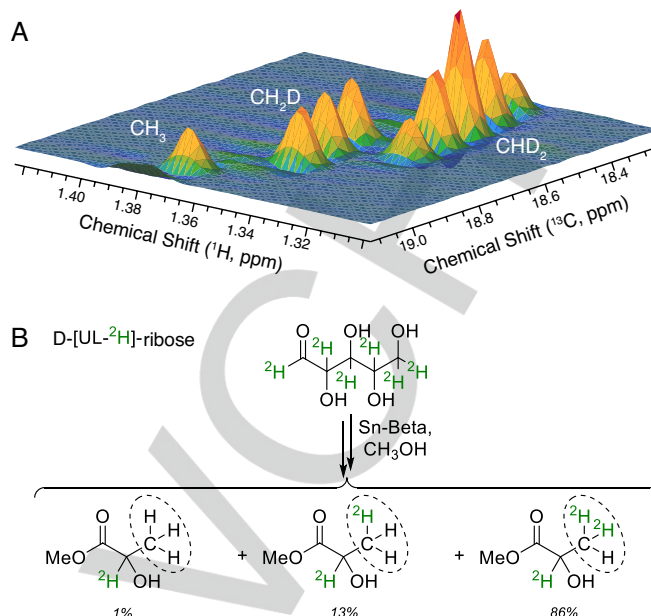


Figure 3. Multiplet pattern of the C3 position of ML, resulting from incorporation of three, two and one ¹H atoms (from left to right), respectively (A). Fraction of lactate isotopomers produced upon reaction of D-[UL-²H₆]-ribose (B). Reactions consisted of 36 mg D-[UL-²H₆]-ribose, 18 mg Sn-Beta, 500 μ L methanol and 5 mg dimethyl sulfoxide (internal standard), which were heated to 160°C for 2 h.

The resulting distribution of ML isotopologues from these experiments is shown in Figure 3B. Most notably, the lack of solvent exchange at the C2 position of lactate supports the hydride shift mechanism in step e (Figure 2) which must therefore be irreversible, as must the enolization in step a. The predominant isotopologue (86%, Figure 3B) contains a single solvent proton at the C3 position, which is consistent with a reversible tautomerization in step c. The predominance of the singly C3 protonated isotopologue (86%) over both the double (13%) and triple (1%) protonated forms indicate that reaction d is significantly faster than the competing reversible tautomerization in c.

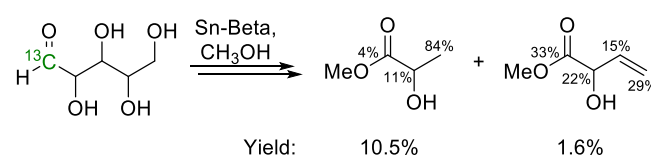


Figure 4. Distribution of ¹³C in methyl lactate and methyl vinyl glycolate starting from D-[1-¹³C]-xylose. Reactions consisted of 36 mg D-[1-¹³C]-xylose, 18 mg Sn-Beta, 500 μ L methanol and 5 mg dimethyl sulfoxide (internal standard), which were heated to 160°C for 5 min. Yields were determined by ¹H-NMR and are given in mol% carbon.

Subsequently, the reaction was repeated using D-[1-¹³C]-xylose, resulting in ¹³C distributions of ML and MVG shown in Figure 4. The prevalence of ¹³C incorporation into the C3 position of ML shows that the C3 position of ML predominantly originates from the C1 position of the substrate. This observation was surprising as the formation of a C3 fragment containing the pentose C1 position could be expected to proceed from a freely formed triose, isomerizing between the aldose and ketose forms. Evidently, such a triose does not emerge in free form at

noteworthy amounts, as it would imply a symmetric ^{13}C distribution in ML as well. We hypothesize that instead of a free triose, the retro-aldol cleavage of a ketose results in an enol covalently bonded to the tin-site, as displayed in Figure 5B. In this form, the primary alcohol arising from the C1 position of the carbohydrate substrate may coordinate and subsequently dehydrate. In this manner, the labeled carbon atom is now positioned in the C3 position of ML, Figure 5B. Notably, the conversion of the carbohydrate C1 position to the lactate C3 position is a common feature of chemocatalytic and biocatalytic glycolysis, resulting from coordination or phosphorylation of the carbohydrate C1 site, respectively.^[27]

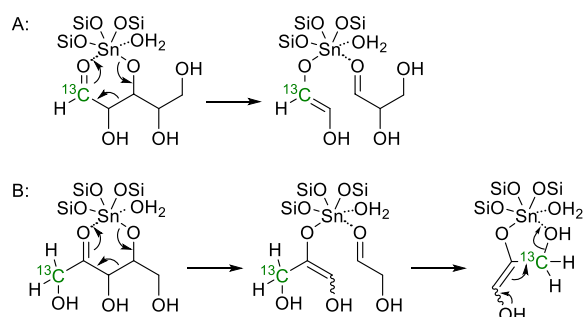


Figure 5. Plausible mechanism for retro-aldol cleavage of a [1- ^{13}C]-aldopentose (A) and a [1- ^{13}C]-ketopentose (B) to glycolaldehyde and glyceraldehyde.

Use of D-[1- ^{13}C]-xylose shows 84% of ^{13}C label at the C3 position of the ML formed, while, ^1H NMR shows 10.5% combined yield of all ML isotopomers and 7.4% yield of the ^{13}C labeled methyl-[3- ^{13}C]-lactate isotopomer, confirming that this isotopomer covers the majority of the methyl lactate formed (Figure S1). These distributions corroborate that the majority of methyl lactate appears to derive from the ketose form (Figure 5B) rather than the aldose (Figure 5A), even when an aldose is used as the substrate. Overall, isotope-tracking data thus suggest that the Sn-Beta catalyzed ML formation predominantly proceeds from the ketose form in a concerted manner where retro-aldol fragments remain bound to the active site. Similar isotope distributions with more than 80% of ^{13}C label at the C3 position of the ML formed are found for shorter (one minute) or longer (2 hours) reaction times.

Upon retro-aldol cleavage of a ketose or aldose to form ML, an equivalent amount of glycolaldehyde is formed, of which the majority must be unlabeled to correspond to the labeled ML formed. MVG may be formed from aldol condensation of two glycolaldehyde fragments. As expected, MVG was detected in the mixture and found to have a much more even distribution of ^{13}C than ML, Figure 4B, with no predominant labeling at any single position.

Methyl 2,5-dihydroxy-3-pentenoate

Recently, it has become clear that various other alpha-hydroxy esters in addition to ML can be formed in significant amounts under similar catalytic conditions. The most abundant of these longer alpha-hydroxy esters formed from pentoses is methyl *trans*-2,5-dihydroxy-3-pentenoate (*trans*-DPM) which can be formed in yields above 30%. *trans*-DPM and ML are five- and three-carbon alpha-hydroxy esters, respectively, and could be

expected to follow similar mechanistic steps in the formation of the alpha-hydroxy ester functionality. The proposed mechanism for *trans*-DPM formation is expected to differ from that of ML shown in Figure 2, in step c. Here, tautomerization is replaced by an additional dehydration step, after which the pathway again can proceed equivalently to the reactions of step d and e for ML. In order to investigate the mechanism for *trans*-DPM formation, we performed an analysis of *trans*-DPM using [UL- $^2\text{H}_6$] and [1- ^{13}C] labelled pentoses equivalent to that performed for ML.

Experiments with the deuterated pentose showed less than 1% total solvent proton incorporation into *trans*-DPM, all of which was observed at the C5 position (Figure 6A). Most importantly, this finding excludes the significance of tautomerization to a free 3-deoxyxylosone type intermediate, as has been previously suggested, in the route to *trans*-DPM.^[14, 20] The same lack of solvent exchange was observed in the pathway to furfural (Figure S2), showing that a 3-deoxyxylosone type intermediate was not part of the pathway for Sn-Beta catalyzed furfural formation.

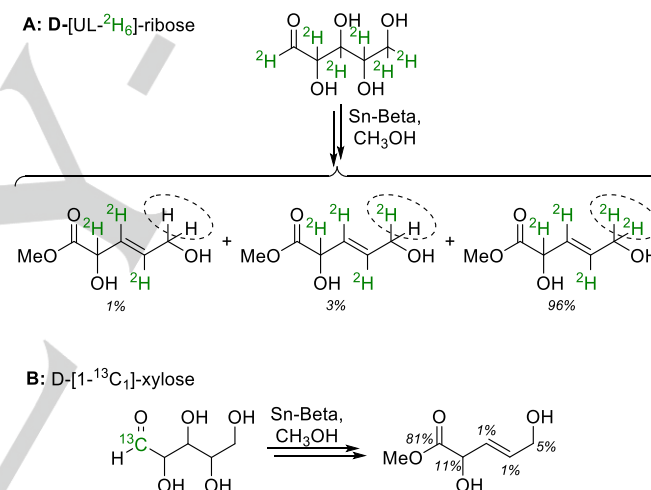


Figure 6. Amount of *trans*-DPM produced with the displayed isotopic configuration upon reaction of D-[UL- $^2\text{H}_6$]-ribose (A). Distribution of ^{13}C in *trans*-DPM using D-[1- ^{13}C]-xylose (B). Reactions consisted of 36 mg substrate, 18 mg Sn-Beta, 500 μL methanol and 5 mg dimethyl sulfoxide (internal standard), which were heated to 160°C for 5 min.

When using the [1- ^{13}C] pentose substrate, the ^{13}C isotope distributions show a peculiar pattern, Figure 6B. No scrambling of ^{13}C isotope into the C3 and C4 positions is observed and these positions retain close to the natural isotopic abundance of ^{13}C . In addition, scrambling of ^{13}C isotope of [1- ^{13}C] pentose into the C2 and C5 positions is observed. Such a scrambling of ^{13}C 1 to C2 and C5 is consistent with epimerization of the carbohydrate through a 1,2-carbon shift or isomerization by a 1,5-hydride shift, respectively. Previously, epimerization by 1,2-carbon shifts had been reported for Sn-Beta at lower temperatures and 1,5-hydride shifts had been reported for other catalysts.^[13, 28-29] In addition, *trans*-DPM is formed as the major isomer in all experiments with negligible formation of the *cis*-isomer. The same selectivity is observed for both Sn-Beta and the homogeneous SnCl_4 , figure S3, thus excluding spatial confinement as the origin of *cis*-*trans*-selectivity.

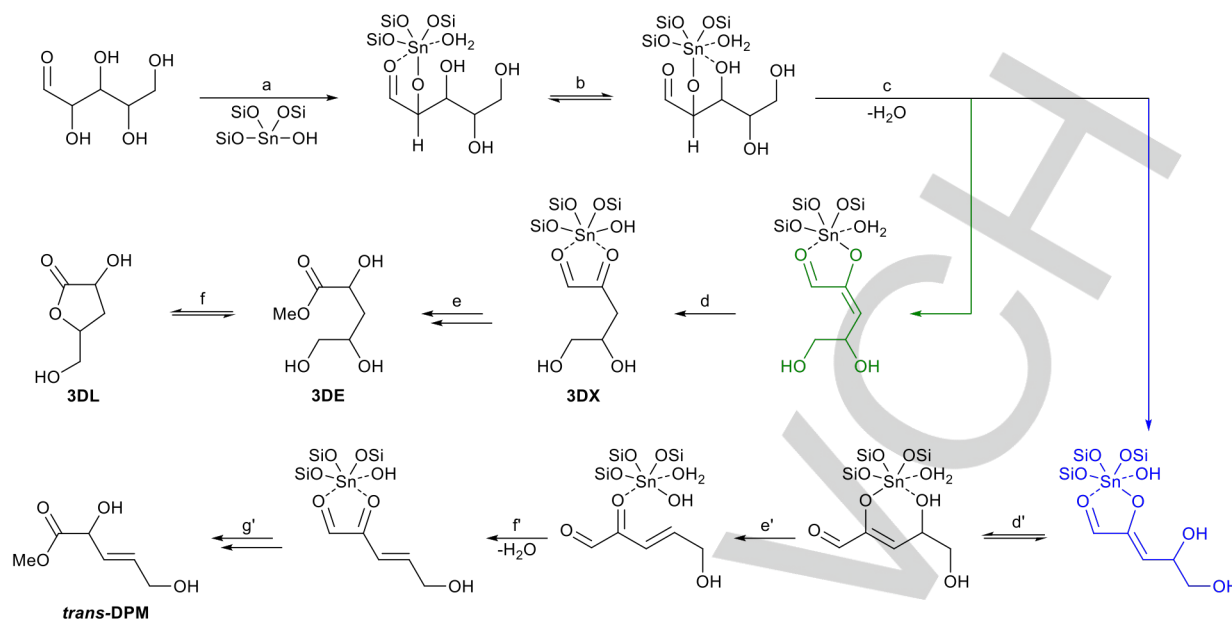


Figure 7. Proposed mechanism for the formation of methyl *trans*-2,5-dihydroxy-3-pentenoate (*trans*-DPM), 3-deoxy- γ -xylonolactone (3DL) and methyl 3-deoxy-xylonate (3DE) from D-xylulose. The *cis*-precursor to step **f'** may also react by alternative pathways to form furfural in addition to the three products displayed.

Based on these observations a plausible reaction mechanism towards the formation of *trans*-DPM is shown in Figure 7 (blue). The C3 position of *trans*-DPM is maintained as a olefinic group throughout. Reversible keto-enol tautomerization of the C3 or C4 positions en route to *trans*-DPM can thus be excluded, as no solvent exchange is found at these positions. Formation of a *trans* C2-C3 bond in step **c** precludes cyclization and leads to a linear pathway forming *trans*-DPM, if a *trans* olefinic bond is formed in step **e'** as well. Formation of a *cis* olefinic bond in step **e'** may enable a cyclic dehydration pathway to furfural, supported by observed furfural isotopologues (Figure S2). Similar to the retro-aldol cleavage of ketose substrates (Figure 5), coordination at C2 elicits most central reaction chemistry in the pathway. Coordination at C2 is vital for hydride shifts in the equilibration of ketose and aldose substrates as well as the dehydration in steps **c** and **e'**.

Figure 7 accounts for the finding that experimentally observed products 3-deoxy- γ -xylonolactone (3DL) and methyl 3-deoxy-xylonate (3DE) derive from the same pathway (green), while *trans*-DPM derives from a different pathway, as witnessed from different patterns of isotope incorporation. Previous experiments identified 3-deoxyglycosone (the glucose analogue of 3DX) in reactions from glucose, and further experiments using a 3-deoxyglycosone substrate had indicated that this substrate predominantly forms saturated lactones and esters, thus supporting steps **e** and **f**.^[14] A rehydration of *trans*-DPM to 3DE seems unlikely as isolated *trans*-DPM does not react to 3DE under the current reaction conditions (Figure S4).

We hypothesize that the flux of intermediates into the two different pathways, for *trans*-DPM (and possibly furanics) as well as 3DL/3DE, may be influenced by the stereochemistry of step **c**. Only the formation of a *trans*-2,3-enol favors subsequent 2,4 coordination and dehydration at C4. Formation of a *cis*-enol in step **c** of Figure 7 (green) will make the simultaneous

coordination of the enol and the C4 hydroxyl group less favorable. In such a case tautomerization to the experimentally observed 3-deoxyxylosone intermediate (3DX) may occur in favor of dehydration, as shown in step **d**, leading to formation the products 3DL and 3DE by intra- or intermolecular esterification, respectively. Experiments with the commercially available α -hydroxy- γ -butyrolactone, showed that under the tested reaction conditions the lactone and the ester equilibrate (Figure S5), demonstrating that the products 3DL and 3DE and their formation is indistinguishable under the tested reaction conditions.

Considering the stereoselectivity for *trans*-DPM formation and previously reported stereoselectivity in the aldose-ketose isomerization by C1-C2 hydride shift,^[30] we set out to identify further stereoselective reactions in the pathways of Figure 7. Experiments with [UL-²H₆] aldopentose showed additional unanticipated isotope distributions in both 3DL and 3DE. Stereoselectivity was observed for the 4 potential diastereomers of 3DL with isomerism at C2 and C3 (Figure 8). While stereoselectivity at the C2 position was low (9% *diastereomeric excess* (*de*) between xylono- and arabino-lactone forms), stereoselectivity for ¹H incorporation at the C3 position by tautomerization was 46% \pm 1% *de* for these two lactones. The same trend was observed for 3DE with 7% *de* for C2, and 46% *de* for both pairs of C3 diastereoisomers (Figure S6). The almost identical stereoselectivity at C2 and C3 in 3DL and 3DE is consistent with the two molecules arising from the same reaction pathway. The high diastereoselectivity of tautomerizations in this pathway and the high *trans* stereoselectivity in the dehydration pathway (Figure 7) parallel the high stereoselectivity in the hydride shifts of aldose-to-ketose isomerizations. Thus, Sn-Beta catalyzes a variety of stereoselective isomerization and dehydration reactions.

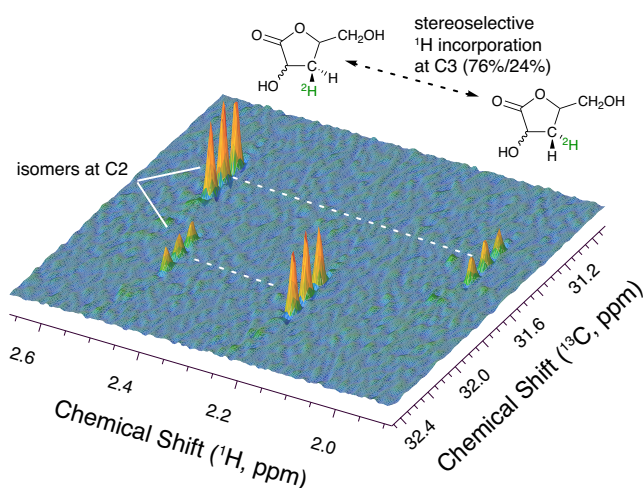


Figure 8. ^1H - ^{13}C -HSQC signals for the C3 position of 3DL. The 4 potential diastereomer products (isomerism at C2 and C3) produced by reaction of enantiomerically pure $[\text{UL-}^2\text{H}_6]$ pentose yield four triplets for CH groups at C3, as splitting by the quadrupolar ^2H was not decoupled.

C5-C1 Hydride shift

To elucidate the ability of Sn-Beta to perform a 5,1-hydride shift $[\text{5,5}'\text{-}^2\text{H}_2]$ -arabinose was selected for testing. Reaction of the $[\text{5,5}'\text{-}^2\text{H}_2]$ -arabinose showed a final distribution of 80% $^2\text{H}_2$ and 20% $^2\text{H}^1\text{H}$ in the C5 position of *trans*-DPM (Figure 9). Compared with the lower (<4%) proton incorporation in the uniformly deuterated substrate (Figure 6A) under identical reaction conditions, this finding indicates that intramolecular hydride exchange is taking place. A C5-C1 hydride shift as previously detected in titanium-Beta catalyzed reactions is the most plausible explanation.^[29] Analysis of the distribution of ^2H in ML from $[\text{5,5}'\text{-}^2\text{H}_2]$ -arabinose showed that the C3 position had 78% $^1\text{H}_3$ and 22% $^2\text{H}^1\text{H}_2$ (Figure S7). This incorporation correlates to migration of the label from the C5 position to the C1 as detected from the isotope pattern in *trans*-DPM after a hydride shift for approximately 20% of the molecules (Figure 9). Thus, use of a $[\text{5,5}'\text{-}^2\text{H}_2]$ labelled pentose underlines that ML primarily originates from the ketose as shown in Figure 5. In addition, the results indicate that a C5-C1 hydride shift occurs in the carbohydrate form, but only for a minority of the substrate carbohydrate.

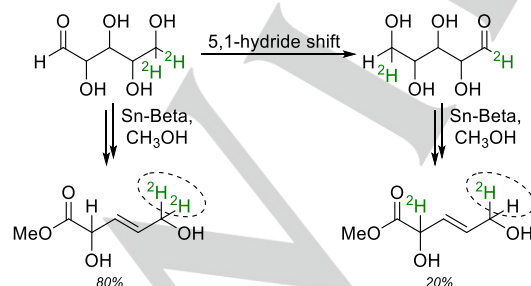


Figure 9. Amount of *trans*-DPM produced with the displayed isotopic configurations upon reaction of a $[\text{5,5}'\text{-}^2\text{H}_2]$ -arabinose. Reactions consisted of 36 mg substrate, 18 mg Sn-Beta, 500 μL methanol and 5 mg dimethyl sulfoxide, which were heated to 160°C for 5 min.

Conclusion

In conclusion, we employ isotope labeled substrates to track isotope redistribution and solvent exchange in varying products formed during chemocatalytic carbohydrate conversion. The experiments have shown that ketoses are the primary substrates for retro-aldol cleavage much like enzymatic glycolysis. This finding is consistent with a rapid hydride-shift between aldose and ketose under reaction conditions of alpha-hydroxy ester formation ($\sim 160^\circ\text{C}$). Furthermore, a strong binding is observed between substrate and catalyst, resulting in regioselective formation of methyl lactate, occurring through irreversible formation of an enol, which continues in a cascade reaction through to methyl lactate. Analogous conclusions can be drawn for pathways that do not encompass a retro-aldol step. Also here, experimental data is consistent with a strong coordination of the enol form by the catalyst active site. These pathways include enols formed in the cascade toward *trans*-DPM, 3DL, 3DE and similar 3-deoxy esters which exhibit little isotope scrambling once the reaction cascade commences. However, our experiments show some atom rearrangement attributed to reactions with unconverted carbohydrates. Sn-Beta was shown to catalyze a 5,1-hydride shift of carbohydrates, as well as previously reported 1,2-hydride shift and a Bilik-type 1,2-carbon shift. We thus find that rich mechanistic information can be derived by use of quantitative isotope-tracking experiments in complex reaction mixtures formed in biomass conversion.

Experimental Section

D- $[\text{1-}^{13}\text{C}]$ -xylose (99% atom-% ^{13}C) was obtained from Sigma-Aldrich. D- $[\text{UL-}^2\text{H}_6]$ and D- $[\text{5,5}'\text{-}^2\text{H}_2]$ -arabinose (98% atom-% ^2H) were obtained from Omicron Chemicals. Reactions were conducted with a Biotage Initiator⁺ microwave reactor in 500 μL glass reaction vials. Reactions were typically conducted with 18 mg Sn-Beta (Si/Sn = 200, hydrothermally synthesized), 36 mg substrate, 500 μL methanol and 5 mg dimethyl sulfoxide as an internal standard.

The Sn-Beta catalyst was hydrothermally synthesized in accordance with the procedure by Tolborg *et al*, employing a target Si/Sn ratio of 200.^[31] The catalyst structure and composition was confirmed by ICP-OES (0.9 wt% Sn, Si/Sn = 196), XRD (*BEA framework) and N_2 absorption ($S_{\text{BET}} = 540 \text{ m}^2 \text{ g}^{-1}$, $S_{\text{micropore}} = 436 \text{ m}^2 \text{ g}^{-1}$, $V_{\text{total}} = 0.30 \text{ mL g}^{-1}$, $V_{\text{micropore}} = 0.22 \text{ mL g}^{-1}$ calculated by the *t*-plot method).

NMR isotope tracking was performed using spectra that were acquired on an 800 MHz Bruker Avance III NMR spectrometer equipped with a TCI CryoProbe and a SampleJet sample handler. Data were zero-filled to double the number of the acquired complex data points prior to Fourier transformation in all spectral dimensions using Bruker Topspin 3.5 p17 software. Quantifications were obtained from qNMR experiments without pulse sequence elements that enhance or suppress signal, but with extensive inter-scan recycle delays. Two-dimensional ^1H - ^{13}C NMR HSQC spectra validated the assignment of the integrated 1D ^{13}C spectra. Incorporation of deuterium was verified from chemical shift changes and multiplet patterns in

HSQC spectra, where spectral editing was used to distinguish carbon sites by the number of attached protons (and hence incorporated deuterons; see Figures 3 and 8). Quantitative proton decoupled ^{13}C 1D spectra were acquired with recycle delays of 60 seconds using a pulse sequence that employs inverse gated decoupling to avoid nuclear Overhauser enhancement of protonated sites.

Acknowledgements

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Keywords: alpha-hydroxy esters • carbohydrates • heterogeneous catalysis • NMR spectroscopy • reaction mechanisms

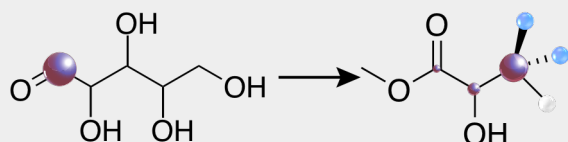
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FULL PAPER



Samuel G. Elliot, Esben Taarning,*
Robert Madsen, and Sebastian Meier*

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**NMR Isotope Tracking Reveals
Cascade Steps in Carbohydrate
Conversion by Sn-Beta**

Pathfinder: Reaction pathways in biomass valorisation often remain enigmatic. Carbohydrates with different isotope composition (different neutron content) were used to elucidate mechanistic details in the chemocatalytic conversion of carbohydrates by Sn-Beta. Results indicate strong coordination of reaction intermediates and mechanistic similarities to enzymatic glycolysis.