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Catalytic Carbohydrate Epimerization

Epimerization of Glycal Derivatives, by a Cyclopentadienyl-ruthenium Catalyst: Application to Metalloenzymatic DYKAT

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Abstract: Epimerization of a non-anomeric stereogenic center in carbohydrates is an important transformation in the synthesis of natural products. In this study an epimerization procedure of the allylic alcohols of glycals, by cyclopentadienylruthenium catalyst **1**, is presented. The epimerization of 4,6-*O*-benzylidene-D-glucal **4** in toluene is rapid and an equlibrium with its D-allal epimer **5** is established within 5 min at room temperature. Exchange rates for allal and glucal formation were determined by 1D ^{1}H EXSY NMR experiments to 0.055 s^{-1} and 0.075 s^{-1}, respectively. For 4-*O*-benzyl-L-rhamnal **8** the epimerization was less rapid and three days of epimerization was required to achieve equilibration of the epimers at room temperature. The epimerization methodology was subsequently combined with acylating enzymes in a dynamic kinetic asymmetric transformation (DYKAT), giving stereoselective acylation to the desired stereoisomers **12**, **13** and **15**. The net effect of this process is an inversion of a stereogenic center on the glycal and yields ranging from 71% to 83% of the epimer were obtained.

Introduction

Bacterial polysaccharides and other natural products commonly contain rare carbohydrates with different stereochemistry compared to the most common monosaccharides. Such molecules are synthetically accessible through stereocontrolled *de novo* synthesis[1], [2] or modifications of readily available carbohydrates.[3], [4] The latter methodology frequently involves stereochemical inversions of secondary alcohols, which regularly follow two-step procedures: triflation followed by nucleophilic displacement or oxidation and subsequent stereoselective reduction.[5], [6], [7] An alternative method would be to use a dynamic kinetic asymmetric transformation (DYKAT) approach utilizing a redox epimerization catalyst to epimerize the alcohol and subsequently trap the inverted alcohol in a transesterification reaction catalyzed by a stereoselective enzyme (Figure 1).

The enzymatic selectivity in a transesterification of secondary alcohols is governed by the size of the substituents residing at the stereogenic center. Assuming that the largest group has a higher priority than the medium group according to the CIP rule, lipases show (*R*)-stereoselectivity, whereas serine proteases show (*S*)-stereoselectivity.[8], [9]

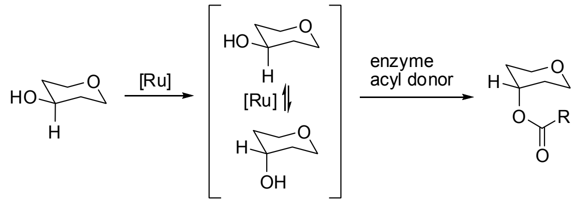

Figure 1. Schematic picture of the epimerization of a carbohydrate molecule and the subsequent trapping of one epimer by an enzyme-catalyzed stereoselective acylation.

Chemoenzymatic dynamic kinetic resolution (DKR) and DYKAT have been applied in asymmetric synthesis of a wide range of secondary alcohols and primary amines with various transition metal complexes as racemization catalysts (examples are given in Figure 2).[10] Of these catalysts, cyclopentadienylruthenium complex **1**, activated by *t*-BuOK, is one of the most efficient catalysts for racemization of *sec*-alcohols[11] and it has been used in combination with both (*R*)-selective lipases[11] and (*S*)-selective proteases.[12] Catalyst **1** has been thoroughly investigated with both IR and NMR techniques giving valuable insight into its activation and catalytic mechanisms.[13],[14]

Transition metal-catalyzed epimerization of secondary alcohols in carbohydrates, utilizing catalyst **1**, has been studied previously.[15] By subjecting partially protected glucose, mannose

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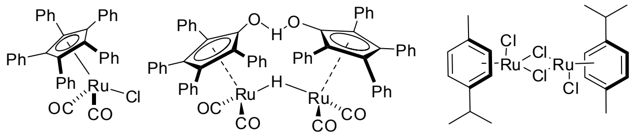


1 2 3

Figure 2. Three examples of ruthenium-based racemization catalyst used in combination with enzymes in DKR protocols.

and allose derivatives to activated **1** in *p*-xylene at 120 °C, it was possible to determine the thermodynamic equilibrium between the non-anomeric epimers of the carbohydrates. Typically, catalyst **1** is able to epimerize secondary alcohols at ambient temperature at a rapid rate; however in the case of these protected carbohydrates, where the secondary alcohol is in close proximity to several electron-withdrawing groups the oxidation step of the racemization is much harder to achieve. Moreover, the neighboring groups increase the bulk around the secondary alcohol, which further decreases the efficiency of the epimerization. We envisioned that by performing the epimerization on a protected glycal derivative, the influence of both of these factors would be lowered and thereby enable a more rapid epimerization. With a faster reaction rate it would be possible to study the epimerization by NMR spectroscopic methods. Furthermore, lower temperatures would allow combination of the transition metal-catalyzed epimerization with an enzymatic resolution, thus giving a DYKAT that would yield an inversion of a stereogenic center.

Results and Discussion

Epimerization

Initially, a suitable racemization catalyst was needed and therefore screening of ruthenium-based catalysts **1** – **3** was conducted. All three catalysts have previously been used successfully in combination with enzymes yielding enatioenriched allylic acetates.[16] Epimerization experiments of 4,6-*O*-benzylidene-D-glucal, **4**, employing catalysts **2** and **3**, under standard conditions, only showed traces of epimerized products after 18 h at 60 °C.[17] Compound **1**, on the other hand, was able to rapidly epimerize **4** at room temperature: aliquots retrieved and analyzed by ^{1}H NMR spectroscopy after 30 and 60 minutes showed a 2:1 mixture of the glucal and allal derivatives **4** and **5** (K_{eq} = 0.504 for **4** ⇋ **5**).

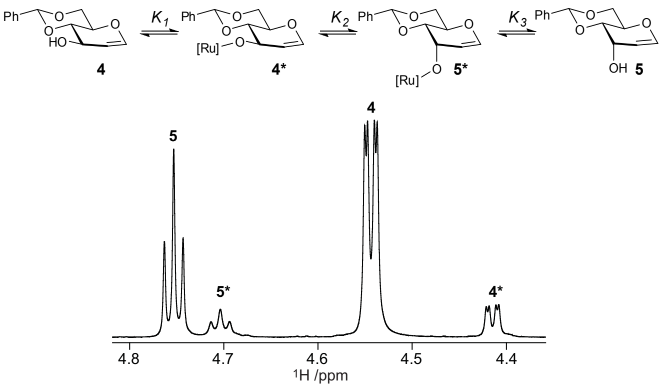


Figure 3. Equilibrium between compounds **4** and **5** using 10 mol% of **1**; part of the ^{1}H NMR spectrum at 25 °C of the equilibrium mixture, showing the H2 resonances. Peaks arising from the catalyst-substrate complexes ([Ru] = catalyst **1**) are marked with asterisks (*). $K_1 \cdot K_3$ = 0.90, K_2 = 0.56.

The rapid establishment of an equilibrium inspired an investigation of the reaction kinetics by NMR spectroscopy. Upon treatment of compound **4** with 10 mol% of activated catalyst **1** in toluene-d_8 solution, the equilibrium between **4** and **5** was established within five minutes at 25 °C. In addition to the C3-epimer, allal **5**, the catalyst-substrate complexes[18] **4*** and **5*** were observed in the ^{1}H NMR spectrum (Figure 3), enabling the determination of the equilibrium constant for the equilibrium between **4*** and **5*** (K_2 in Figure 3). Subsequent additions of activated catalyst did not increase the complex concentration significantly.

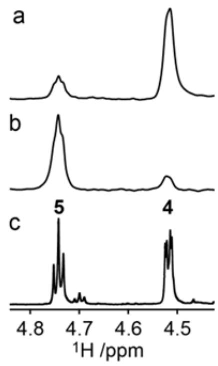
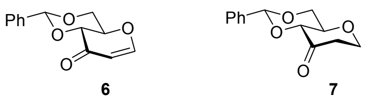


Figure 4. Equilibrium between **4** and **5**, facilitated by treatment with *t*-BuOK activated **1** in toluene-d_8 solution at 90 °C and detected by: a) 1D ^{1}H EXSY NMR with selective excitation of the H2 resonance at 4.52 ppm in **4**, b) 1D ^{1}H EXSY NMR with selective excitation of the H2 resonance at 4.74 ppm in **5** and c) the corresponding 1D ^{1}H NMR spectrum

At a sample temperature of 90 °C, the ratio between compounds **4** and **5** was 4:3 and the equilibrium constants were determined to $K_1 \cdot K_3$ = 0.71 and K_2 = 1.10 by ^{1}H NMR. 1D ^{1}H EXSY NMR experiments [19] were performed at 90 °C with selective excitation of the H2 signals of epimers **4** and **5**. With a 3 s mixing period exchange peaks were obtained (Figure 4), which showed that a fast equilibrium between **4** and **5** occurred and enabled establishment of the exchange rates. Full exchange matrix analysis gave the exchange rates k_+ = 0.055 s^{-1} and k_- = 0.075 s^{-1} (Table 1). No significant exchange peaks were observed at lower temperatures. At elevated temperatures ^{1}H NMR data showed a buildup of two ketones: **6**[20] and its suggested saturated analog **7** (Figure 5); both irreversibly formed through oxidation and redox isomerization processes, respectively.[21]



6 7

Figure 5 Ketones formed at elevated temperature in the epimerization reaction of compound **4**.

Epimerization of the 6-deoxygenated compound, 4-*O*-benzyl L-rhamnal **8**, proceeded at a much lower rate. Equilibration of the epimers **8** and **9** required 4 days of reaction time at room temperature (Figure 6). Rate constants for the epimerization could be extracted through a least-sum-square fitting of the experimental concentrations at different times to a kinetic model. The kinetic model is based on first order kinetics regarding the disappearance and formation of **8** and **9**, respectively (Table 1).

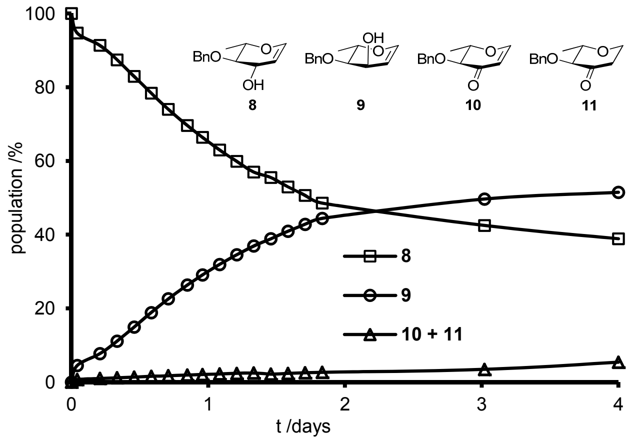


Figure 6 Plot of the epimerization equilibrium between **8** (□) and **9** (○) after treating **8** with *t*-BuOK-activated **1** (10 mol%) in toluene-d_8 solution at 25 °C. Oxidation by-products **10** + **11** (Δ) are formed in small amounts. Solid lines are drawn through the data points as an aid to the eye. Populations are obtained by integration of ^{1}H NMR spectra.

Table 1. Exchange rates and energies of the epimerizations of **4** at 90 °C and of **8** at 25 °C.

Reaction	K_{eq}[a]	k_+ /10^{-3} (s^{-1}) [b]	k_- /10^{-3} (s^{-1}) [b]	$\Delta G^‡$ (kJ mol^{-1})
4 ⇋ **5**	0.77	55	75	95.7
8 ⇋ **9**	1.3	0.13	0.086	93.9

[a] Determined by integration of the epimers peaks in ^{1}H NMR.

Notably, catalyst-substrate complexes were not observed in the ^{1}H NMR spectrum of the reaction mixture, suggesting that the Ru-alkoxide complexes of **8** and **9** are less favored compared to the free Ru-OtBu complex. Disfavored formation of the Ru-alkoxide complexes may be ascribed to steric bulk created by the adjacent freely rotating protecting group that could interfere in the interaction between the ruthenium and hydroxyl group. The equilibrium mixture consisted of **8** and **9** in a 1:1.3 ratio, accompanied by 10% of ketones **10** and **11** combined.

Ring conformation and equilibrium

The D-glycals, compounds **4** and **5** were assigned to occupy the $^4H_5$ conformation,[22] while the 6-deoxy-L-glycals **8** and **9** occupy the $^5H_4$ conformation; in both cases this results in an antiperiplanar relationship between the axially oriented H4 and H5 atoms. The conformational assignments rely on the large $J_{H4,H5}$ coupling constants (9.6 – 10.2 Hz) and are supported by previous reports on similar compounds (Figure 7). [23] Compound **4**, in which the OH group is equatorially oriented, dominated the equilibrium between **4** and **5**, while compound **9**, in which the OH group is axially oriented, was the most populated epimer in the equilibrium between **8** and **9**.

In compound **9** the OH proton displayed signs of hydrogen bonding in the ^{1}H NMR spectrum: The downfield chemical shift (δ_{HO}(**9**) ≫ δ_{HO}(**8**)) and coupling constant J_{HOCH} = 3.1 (Table 2)

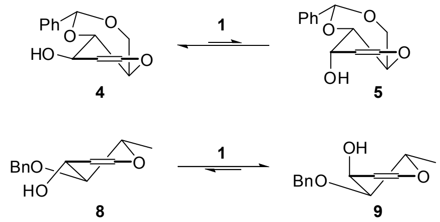


Figure 7. Equilibria of compounds **4** and **5**, and **8** and **9**, respectively, under catalysis of **1**. The dihydropyran rings are schematically depicted in their preferred conformations.

indicates hydrogen bonding to the benzyl ether oxygen.[24] Analysis of molecular models of compounds **8** and **9** revealed a favorable geometry for hydrogen bonding in the latter compound, but not in the former. An intramolecular hydrogen bond could thus stabilize epimer **9**. This hypothesis was tested by adding a competitive hydrogen bond donor (*t*-BuOH) to the epimerization reaction, that potentially could interfere with this internal hydrogen bond. When *t*-BuOH was added the equilibrium shifted towards epimer **8** (to give K_{eq} = 1.08) which further supports the existence of an intramolecular hydrogen bond in **9**.[25] A similar trend, although of smaller magnitude, is present in compounds **4** and **5**, where δ_{HO}(**5**) > δ_{HO}(**4**). The coupling constant J_{HOCH} = 1.9 Hz in **5** indicates restricted motion around the C3-O3 bond, with a probable alignment towards O4 since the distance to the OH group is short in the molecular model.

Table 2. Experimental chemical shifts and coupling constants in ppm and Hz, respectively, and interatomic distances from molecular models, in Å, of the glycal hydroxyl groups.

	4	*5*	*8*	*9*
δ_{HO3}	1.434 ppm	1.991 ppm	1.130 ppm	2.113 ppm
$^3J_{H3,HO3}$	4.51 Hz	1.87 Hz	6.38 Hz	3.09 Hz
$r_{O4 \cdots HO3}$	2.6 Å	2.1 Å	3.1 Å	2.4 Å

Dynamic kinetic asymmetric transformation

Inspired by the efficient redox epimerization of glycals **4** and **8** at moderate temperatures we were interested in achieving a DYKAT by coupling this process with a stereoselective biocatalyst. By choosing an (*R*)-selective lipase, favoring the transesterification of L-rhamnal epimer **9**, an inversion of the C3 alcohol stereocenter would be obtained (Figure 8). Owing to its known stability and activity over prolonged reaction times the lipase *Candida Antartica* Lipase B, CALB, was considered suitable as a transesterification catalyst.

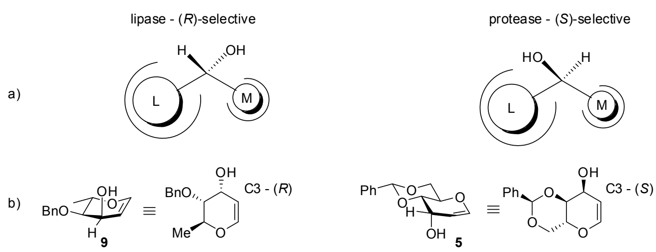


Figure 8. a) Empirical enantiopreference for a lipase and a protease. L = Large-sized substituent, M = Medium-sized substituent. b) Glycals preferred in transesterifications.

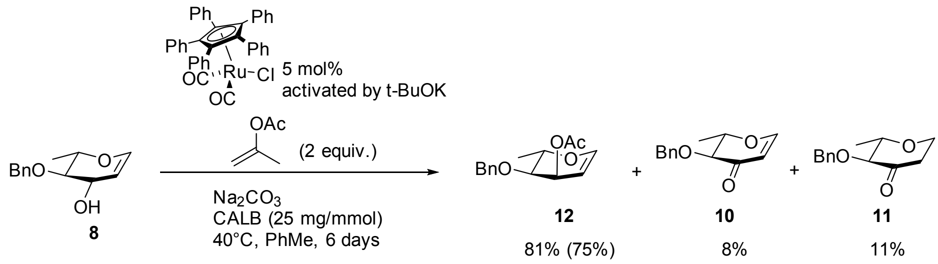


Figure 9. Dynamic kinetic resolution of L-rhamnal **8** (isolated yield in parenthesis).

The biocatalyst was mixed with *t*-BuOK-activated **1**, isopropenyl acetate and Na_2CO_3 in toluene at 40 °C and the reaction reached full conversion after 6 days, transforming 81% (75% isolated yield) of L-rhamnal into **12**.[26] The remaining 19% consisted of ketones **10** (8%) and **11** (11%) (Figure 9). These compounds arise from the ruthenium alkoxide of **8** (or its epimer **9**), where ketone **10** is formed via dehydrogenation and ketone **11** via isomerization.[21] Notably, enzymatic acylation of (*S*)-alcohol **8** was not observed, showing that CALB is highly selective for transesterification of the corresponding (*R*)-alcohol.

In order to achieve a similar inversion at C3 of the D-glucal derivative, **4**, to acylated D-allal, a lipase is unsuitable since the stereogenic center at C3 already possesses the configuration preferred in a lipase-catalyzed transesterification.[27] This was verified by subjecting **4** to CALB and isopropenyl acetate in dry toluene, which afforded 17% of acetylated product after only 1 h of stirring. Instead, a protease, *Subtilisin Carlsberg*, displaying the opposite stereoselectivity compared to a lipase, was chosen for selective transesterification of the epimer of **4** (*i.e.* **5**). The activity of commercially available *Subtilisin Carlsberg* is low in transesterification reactions, but it can be enhanced by coating the enzyme with the surfactants octyl β-D-glucopyranoside and Brij 58 (polyoxyethylene (20) cetyl ether).[12] These surfactants form a reversed micelle around the enzyme with the concomitant solubilization of small amounts of water, thereby providing a stable aqueous microenvironment.[28] The drawback of using this coating technique is the requirement of a more polar solvent such as THF. Water present in this solvent and in the reversed micelles adds up to give a relatively high water content in the reaction solution, increasing the risk of decomposition of **1** in the DYKAT reaction.

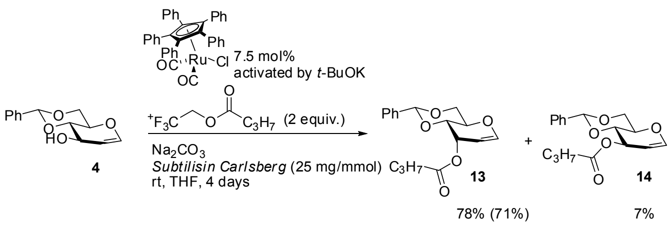


Figure 10. Dynamic kinetic resolution of 4,6-*O*-benzylidene-D-glucal, **4**. The reaction stopped at 85% conversion due to decomposition of the epimerization catalyst. Isolated yield in parenthesis.

Several attempts of DYKAT of **4** were performed at room temperature employing **1** for epimerization; however, the reaction was in all cases terminated before full conversion was reached. The best result was obtained after storing the enzyme preparation together with freshly activated molecular sieves under vacuum for three days, and using 2,2,2-trifluoroethyl butyrate as an acyl donor; thereby reaching 55% conversion (44 % isolated) of butyrate ester **13** after 18 h (Figure 10). At this point only **13** and **4** could be observed by ^{1}H NMR indicating that the enzyme was highly selective for transesterification of **5**, but that the epimerization had ceased. This was further confirmed by adding racemic 1-phenylethanol after 18 h of reaction time and observing acylation with 16% conversion within 2 h. In order to achieve a higher conversion, the epimerization catalyst was added in three portions à 2.5 mol% at 18 h intervals. Utilizing this protocol the conversion of **4** to **13** was increased to 78% (71% isolated) with a small fraction of the glucal ester **14**, arising from a background chemical acylation. About 15% of starting material **4** remained unconverted.

Also the reverse reaction, *i.e.*, the transformation of D-allal **5** to acetylated D-glucal derivative **15**, was performed. CALB was used as acylating enzyme and full conversion was achieved within 18 h of stirring yielding **15** in 83% yield (Figure 11). The possibility of obtaining carbohydrates with both axial and equatorial hydroxyl groups highlights the viability of the protocol and makes it an interesting method for the preparation of various carbohydrate isomers.

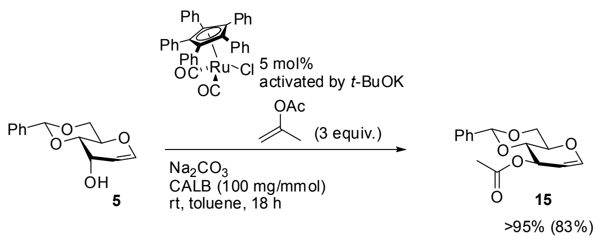


Figure 11. Dynamic kinetic resolution of D-allal **5** (isolated yield in parenthesis).

Conclusion

The epimerization of glycals **4** and **8** with cyclopentadienylrutheium catalyst **1**, studied by NMR spectroscopic methods, was found to be significantly more efficient compared to that of saturated carbohydrates.[15] The epimerization rates varied with the substitution pattern of the glycals; the 4,6-di-*O*-benzylidene-protected compounds (**4** and **5**) reacted faster than the 4-*O*-benzyl-6-deoxy compounds (**8** and **9**). Thanks to the efficacy of the epimerization, which occurred at ambient temperatures, the combination of the epimerization protocol with an enzymatic resolution was possible, resulting in a DYKAT. Due to excellent selectivity for acylation of the axial hydroxyl groups at C3, when employing *Subtilisin Carlsberg* for the D-glycal system **4**/**5** and CALB for the L-glycal system **8**/**9**, we were able to achieve a net inversion of a stereogenic center on the carbohydrate derivatives. Also by changing from *Subtilisin Carlsberg* to CALB the reverse reaction was achieved, transforming axial substrate **5** to its equatorial epimer ester **15**. To the best of our knowledge this is the first DYKAT on glycals of this type. The acyl derivatives **12** and **13** could be employed in syntheses towards α-L-digitoxose-containing natural products[29] such as Jadomycin B[30] and Kijanimicin[31] (from **12**) and allosamidin[32] and α-*C*-glycosides[33] (from **13**).

Experimental Section

General methods

Unless otherwise noted, all reagents and reactants were used as received from commercial suppliers. Isopropenyl acetate was dried over $CaCl_2$ and distilled before use. Dry toluene was obtained from a VAC-solvent purifier system and dry THF through distillation over sodium/benzophenone. All other solvents were obtained from commercial suppliers and used without further purification/drying. For reactions conducted under dry/inert conditions standard Schlenk techniques were used (unless otherwise noted). All reactions were monitored by thin-layer chromatography using E. Merck silica gel 60 F254 plates (TLC analysis). Flash chromatography was carried out with 60Å (particle size 35-70 µm) normal phase silica gel. Optical rotation was measured with a polarimeter equipped with a Na lamp. HR-MS was recorded on an ESI-MS. Ruthenium complex **1** ($\eta^5C_5Ph_5$)Ru(CO)$_2$Cl was synthesized according to a literature procedure.[11] CALB (Novozym 435) and CLEA201-UF is commercially available and surfactant-treated *Subtilisin Carlsberg* was prepared according to know procedures.[12] NMR experiments for conformational analysis were carried out on a Bruker 600 MHz spectrometer equipped with a BBI Z-gradient probe for ^{1}H NMR measurements. Temperatures were calibrated from ^{1}H peak separation of neat ethylene glycol.[34] Chemical shifts were referenced to intrinsic toluene-d_7 (δ_H 7.09). ^{1}H chemical shifts and $^nJ_{HH}$ coupling constants were determined with aid of the PERCH NMR spin simulation software (PERCH Solutions Ltd., Kuopio, Finland). Chemical shifts and coupling constants were altered iteratively until the simulated and experimental spectra appeared highly similar according to visual inspection and the total root-mean-square value was close to or below 0.1%. NMR chemical shift assignments for alcohols **5**[35] and **9**[36] as well as for and ketones **6**[19] and **10**[37] were in agreement with literature data.

General procedure for NMR spectroscopic studies o epimerization mixtures by NMR

Catalyst **1** (6.4 mg, 0.01 mmol) was dissolved in toluene-d_8, 0.1 mL, and activated by treatment with *t*-BuOK (20 µL, 0.5 M in dry THF-d_8), under argon atmosphere. The activation of the catalyst complex was verified by ^{13}C NMR spectroscopy[11] at 25 °C. The diastereomerically pure glycal (0.1 mmol) was dissolved in toluene-d_8, 0.4 mL, and added to the activated catalyst. The sample mixture was entered to the NMR spectrometer, and the system was tuned, matched, locked and shimmed at 25 °C, whereafter a ^{1}H NMR spectrum was recorded. The establishment of equilibrium was monitored by subsequent recordings of ^{1}H NMR spectra.

The 1D ^{1}H,^{1}H-EXSY spectra of compound **4** and **5** were acquired at 90 °C, with a mixing time of 3 s and total relaxation delay of 10.7 s. Exchange matrix analysis of the EXSY spectra was performed by employing the EXSYCalc software, version 1.0 (Mestrelab Research).[38] Three-dimensional models of compounds **4**, **5**, **8** and **9** were built using the Vega ZZ software (release 3.0.1.22).[39]

1,5-anhydro-4-*O*-benzyl-2,6-dideoxy-L-*erythro*-hex-1-eno-3-ulose (10)
^{1}H NMR (CDCl$_3$, 400 MHz) δ: 7.41–7.25 (5H, m, ArH), 7.37 (1H, d, J_{12} 5.9 Hz, H1), 5.38 (1H, d, J_{12} 5.9 Hz, H2), 5.03 (1H, d, J_{gem} –11.5 Hz, C*H*$_2$-Ph), 4.66 (1H, d, J_{gem} –11.5 Hz, C*H*$_2$-Ph), 4.48 (1H, dq, J_{45} 9.8 Hz, J_{56} 6.5 Hz, H5), 3.72 (1H, d, J_{45} 9.8 Hz, H4), 1.43 (3H, d, J_{56} 6.5 Hz, H6); ^{13}C NMR (CDCl$_3$, 100 MHz) δ: 193.0, 162.1, 137.4, 128.42 (2C), 128.41 (2C), 128.0, 105.0, 78.66, 78.65, 73.9, 17.1; HRMS (ESI): calc. for [M+Na] $C_{13}H_{14}NaO_3$: 241.0831, found 241.0835.

1,5-anhydro-4-*O*-benzyl-2,6-dideoxy-L-*erythro*-hex-3-ulose (11)
^{1}H NMR (CDCl$_3$, 400 MHz) δ: 7.39–7.27 (5H, m, ArH), 4.95 (1H, J_{gem} –11.4 Hz, C*H*$_2$-Ph), 4.50 (1H, J_{gem} –11.4 Hz, C*H*$_2$-Ph), 4.20 (1H, ddd, J_{gem} –11.4 Hz, $J_{1pro\text{-}R,2pro\text{-}R}$ 1.4 Hz, $J_{1pro\text{-}R,2pro\text{-}S}$ 7.4 Hz, H1pro-*R*); 3.64 (1H, ddd, J_{gem} –11.4 Hz, $J_{1pro\text{-}S,2pro\text{-}R}$ 2.5 Hz, $J_{1pro\text{-}S,2pro\text{-}S}$ 12.6 Hz, H1pro-*S*), 3.64 (1H dd, $J_{2pro\text{-}S,4}$ 1.3 Hz, J_{45} 9.4 Hz, H4), 3.59 (1H, dq, J_{56} 6.0 Hz, J_{45} 9.4 Hz, H5), 2.71 (1H, dddd, J_{gem} –13.9 Hz, $J_{2pro\text{-}S,4}$ 1.3 Hz, $J_{1pro\text{-}S,2pro\text{-}S}$ 12.6 Hz, $J_{1pro\text{-}R,2pro\text{-}S}$ 7.4 Hz, H2pro-*S*), 2.46 (1H, ddd, J_{gem} –13.9 Hz, $J_{1pro\text{-}R,2pro\text{-}R}$ 1.4 Hz, $J_{1pro\text{-}S,2pro\text{-}R}$ 2.5 Hz, H2pro-*R*), 1.38 (3H d, J_{56} 6.0 Hz, H6); ^{13}C NMR (CDCl$_3$, 100 MHz) δ: 205.8, 137.5, 128.5 (2C), 128.3 (2C), 128.0, 85.3, 78.3, 73.4, 67.0, 42.8, 19.3; HRMS (ESI): calc. for [M+Na] $C_{13}H_{16}NaO_3$: 243.0996, found 243.0092

Dynamic Kinetic Asymmetric Transformations

3-*O*-Acetyl-1,5-anhydro-4-*O*-benzyl-2,6-dideoxy-L-*ribo*-hex-1-enitol (12)
To a flame-dried 5 mL micro-vial were added ($\eta^5C_5Ph_5$)Ru(CO)$_2$Cl **1** (4.75 mg, 0.0075 mmol), Na_2CO_3 (16 mg, 0.15 mmol) and CALB (8 mg). The system was purged with three argon-vaccum cycles. The vial was transferred to an Ar filled glove box and dry, degassed toluene (0.6 mL) was added. *t*-BuOK (15µL, 0.5 M in dry THF, 0.0075 mmol) was added and a color change from yellow to red was observed. After 6 min of stirring the cap was removed and 4-*O*-benzyl-L-rhamnal **8** (33 mg, 0.15 mmol) was added in one portion as a solid and the system was quickly sealed with a teflon septum cap. After an additional 4 min isopropenyl acetate (45 µL, 0.45 mmol) was added and the vial was taken out of the glove box and stirred at 40 °C for 6 days. When full conversion was reached the reaction mixture was filtered through celite with EtOAc and the solvent was subsequently evaporated. The residue was purified by column chromatography (SiO_2, gradient from pentane to pentane/EtOAc 9:1) yielding 29 mg (75%) of **12** in the second fraction.
^{1}H NMR (CDCl$_3$, 400 MHz) δ: 7.38–7.28 (5H, m, ArH), 6.47 (1H, d, J_{12} 5.9 Hz, H1), 5.52 (1H, dd, J_{23} 6.0 Hz, J_{34} 3.7 Hz, H3), 4.87 (1H, dd, J_{12} 5.9, J_{23} 6.0 Hz, H2), 4.71 (1H, d, J_{gem} –11.4 Hz, C*H*$_2$-Ph), 4.45 (1H, d, J_{gem} –11.4 Hz, C*H*$_2$-Ph), 4.08 (1H, dq, J_{45} 10.2 Hz, J_{56} 6.3 Hz, H5), 3.42 (1H, dd, J_{34} 3.7 Hz, J_{45} 10.2 Hz, H4), 2.08 (3H, s, Me), 1.37 (3H, d, J_{56} 6.3 Hz, H6); ^{13}C NMR (CDCl$_3$, 100 MHz) δ: 171.0, 148.4, 137.7, 128.6 (2C), 128.4 (2C), 128.1, 97.3, 77.3, 71.9, 70.5, 62.0, 21.5, 17.8; HRMS (ESI): calc. for [M+Na] $C_{15}H_{18}NaO_4$: 285.1097, found: 285.1089; $[\alpha]^{25}_D$ –163° (c 0.2, CDCl$_3$).

1,5-Anhydro-4,6-*O*-benzylidene-3-*O*-butanoyl-2-deoxy-D-*ribo*-hex-1-enitol (13)
To a flame-dried Schlenk flask was added ($\eta^5C_5Ph_5$)Ru(CO)$_2$Cl **1** (7.9 mg, 0.0125 mmol), Na_2CO_3 (53 mg, 0.5 mmol) and surfactant-treated *Subtilisin Carlsberg* (9 mg). The system was purged with three argon-vaccum cycles. Distilled and degassed THF (1 mL) was added followed by *t*-BuOK (25 µL, 0.5 M in dry THF, 0.0125 mmol) giving rise to a color change from yellow to red. After 6 min stirring 4,6-*O*-benzylidene-D-glucal **4** (117 mg, 0.5 mmol) was added in one portion as a. After an additional 4 min 2,2,2-trifluoroethyl butyrate (150 µL, 1 mmol) was added. Two batches of activated **1** (7.9 mg in 0.2 ml distilled and degassed THF activated by *t*-BuOK as previously) was added after 18 h and 36 h, thereafter the reaction was stirred for additionally 4 h. The reaction mixture was filtered through Celite® with EtOAc, evaporated onto SiO_2 (0.2 g) and purified by column chromatography (4 g SiO_2, eluent pentane to pentane/EtOAc 10:1) yielding 107 mg (71%) of **13** as a white solid.
^{1}H NMR (CDCl$_3$, 400 MHz) δ: 7.49–7.42 (2H, m, ArH), 7.39–7.32 (3H, m, ArH), 6.50 (1H, d, J_{12} 6.0 Hz, H1), 5.61 (1H, s, C*H*Ph), 5.46 (1H, dd, J_{23} 5.9 Hz, J_{34} 4.0 Hz, H3), 5.01 (1H, dd, J_{12} 6.0 Hz, J_{23} 5.9 Hz, H2), 4.46 (1H, dd, $J_{56'}$ 5.3 Hz, J_{gem} –10.7 Hz, H6′), 4.18 (1H, ddd, J_{45} 10.5 Hz, J_{56} 10.3 Hz, $J_{56'}$ 5.3 Hz, H5), 3.98 (1H, dd, J_{34} 4.0 Hz, J_{45} 10.5 Hz, H4), 3.84 (1H, at, J_{56} 10.3 Hz, J_{gem} –10.7 Hz, H6), 2.33 (2H, t, *J* 7.5 Hz, -C*H*$_2$CH$_2$CH$_3$), 1.66 (2H, tq, *J* 7.5 Hz, *J* 7.5 Hz, -CH$_2$C*H*$_2$CH$_3$), 0.93 (3H, t, *J* 7.5 Hz, -CH$_2$CH$_2$C*H*$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz) δ: 173.3, 147.3, 137.2, 129.2, 128.4 (2C), 126.2 (2C), 101.7, 98.7, 76.2, 68.8, 65.1, 61.8, 36.5, 18.6, 13.8; HRMS (ESI): calc. for [M+Na] $C_{17}H_{20}NaO_5$: 327.1195, found 327.1203; $[\alpha]^{25}_D$ +183° (c 0.2, CDCl$_3$).

1,5-Anhydro-4,6-*O*-benzylidene-3-*O*-butanoyl-2-deoxy-D-*arabino*-hex-1-enitol (14)
^{1}H NMR (CDCl$_3$, 400 MHz) δ: 7.52–7.45 (2H, m, ArH), 7.41 – 7.33 (3H, m, ArH), 6.39 (1H, dd, J_{12} 6.1 Hz, J_{13} 1.5 Hz, H1), 5.60 (1H, s, C*H*Ph), 5.56 (1H, ddd, J_{13} 1.5 Hz, J_{23} 2.1 Hz, J_{34} 7.6 Hz, H3), 4.80 (1H, dd, J_{12} 6.1 Hz, J_{23} 2.1 Hz, H2), 4.39 (1H, dd, $J_{56'}$ 4.8 Hz, J_{gem} –10.6 Hz, H6′), 4.04 (1H, dd, J_{34} 7.6 Hz, J_{45} 10.5 Hz, H4), 3.99 (1H, ddd, J_{45} 10.5 Hz, J_{56} 10.2 Hz, $J_{56'}$ 4.8 Hz, H5), 3.85 (1H, dd, J_{56} 10.2 Hz, J_{gem} –10.6 Hz, H6), 2.32 (2H, t, *J* 7.4 Hz, -C*H*$_2$CH$_2$CH$_3$), 1.66 (2H, tq, *J* 7.4 Hz, *J* 7.4 Hz, -CH$_2$C*H*$_2$CH$_3$), 0.93 (3H, t, *J* 7.4 Hz, -CH$_2$CH$_2$C*H*$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz) δ: 173.6, 145.5, 137.1, 129.3, 128.5 (2C), 126.3 (2C), 101.7, 101.1, 77.2, 69.0, 68.7, 68.5, 36.4, 18.6 13.7; HRMS (ESI): calc. for [M+Na] $C_{17}H_{20}NaO_5$: 327.1203, found 327.1191; $[\alpha]^{25}_D$ –83° (c 0.2, CDCl$_3$).

3-*O*-Acetyl-1,5-anhydro-4,6-*O*-benzylidene-2-deoxy-D-*arabino*-hex-1-enitol (15)
To a flame-dried micro-vial were added ($\eta^5C_5Ph_5$)Ru(CO)$_2$Cl **1** (9 mg, 0.015 mmol), Na_2CO_3 (36 mg, 0.15 mmol) and CALB (33 mg). The system was

purged with three argon-vaccum cycles after which degassed toluene (1 mL) was added. *t*-BuOK (32 µL, 0.5 M in dry THF, 0.0016 mmol) was added and a color change from yellow to red was observed. After 6 min of stirring the cap was removed and 4,6-*O*-benzylidene-D-allal **5** (78 mg, 0.33 mmol) was added in one portion as a solid and the system was quickly sealed with a teflon septum cap. After an additional 4 min isopropenyl acetate (110 µL, 1 mmol) was added and the reaction was stirred at 40 °C for 18 h. When full conversion was reached the reaction mixture was filtered through celite with EtOAc and the solvent was subsequently evaporated. The residue was purified by column chromatography (SiO_2, gradient from pentane to pentane/EtOAc 9:1) yielding 76 mg (83 %) of **15** in the second fraction. Experimental data were in accordance with those previously reported.[32] ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.52–7.47 (2H, m, ArH), 7.41–7.35 (3H, m, ArH), 6.39 (1H, dd, J_{12} 6.2 Hz, J_{13} 1.5 Hz, H1), 5.60 (1H, s, C*H*Ph), 5.53 (1H, ddd, J_{34} 7.7 Hz, J_{23} 2.1 Hz, J_{13} 1.5 Hz, H3), 4.81 (1H, dd, J_{12} 6.2 Hz, J_{23} 2.1 Hz, H2), 4.39 (1H, dd, $J_{56'}$ 5.1 Hz, J_{gem} –10.7 Hz, H6′), 4.03 (1H, dd, J_{34} 7.7 Hz, J_{45} 10.5 Hz, H4), 3.99 (1H, ddd, J_{45} 10.5 Hz, J_{56} 10.2 Hz, $J_{56'}$ 5.1 Hz, H5), 3.85 (1H, dd, J_{56} 10.2 Hz, J_{gem} –10.7 Hz, H6), 2.09 (3H, s, Me); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 170.9, 145.6, 137.1, 129.4, 128.5 (2C), 126.4 (2C), 101.8, 100.9, 77.1, 69.03, 69.00, 68.5, 21.4; HRMS (ESI): calc. for [M+Na] $C_{15}H_{16}NaO_5$: 299.0890, found 299.0894; $[\alpha]^{25}_D$ −95° (c 0.8, $CDCl_3$).

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Keywords: Dynamic kinetic asymmetric transformation • carbohydrates • NMR • inversion • enzyme catalysis

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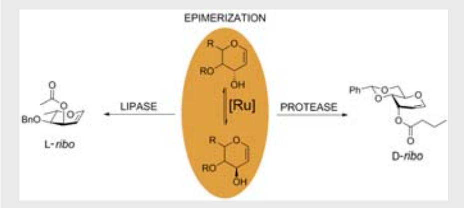
FULL PAPER

Selective inversion of stereogenic centers in carbohydrates is obtained through a dynamic kinetic asymmetric transformation. The latter process is a combination of a ruthenium-catalyzed epimerization and an enantioselective enzyme-catalyzed acylation. The epimerization was studied by NMR spectroscopy, making it possible to determine rates and equilibria of the processes.



Subject Heading

Richard Lihammar, Jerk Rönnols, Göran Widmalm, Jan –E. Bäckvall**

■■ – ■■

Epimerization of Glycal Derivatives, by a Cyclopentadienyl-ruthenium Catalyst: Application to Metalloenzymatic DYKAT