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Chemical versus enzymatic acetylation of α-bromo-ω-hydroxyaldehydes: decyclization of hemiacetals by lipase

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Abstract—Lipase-catalyzed decyclization of hemiacetals of α-bromo-ω-hydroxyaldehydes followed by trapping upon acetylation was observed. Quantum chemical investigations were performed to explain the energetic background of the reactions. The stereocontrolled synthesis of enantiopure *trans*-(2*S*,3*S*)-2-methoxy-tetrahydropyran-3-ol was elaborated.

Deoxysugars play a significant role in living organisms where they are mainly found as subunits of oligosaccharide moieties of glycoconjugates. A number of different deoxymonosaccharides are present in antibiotics and other physiologically active compounds. Because of their pharmaceutical importance, the synthesis of deoxysugars has become an important field of natural product research.[1] Moreover, deoxysugars act as efficient chiral auxiliaries,[2] probably because of the high structural rigidity which is characteristic of carbohydrates and their deoxyanalogs.

On the other hand, studies of activation of (deoxy)-saccharides by lipase probably allowing isomerization, transfer, etc. of these moieties could be useful for understanding the nature of these processes occurring in some systems in vivo.

For the synthesis of alkyl glycosides of some deoxysugars we chose a chemoenzymatic approach (Scheme 1) consisting of three steps:

1. Bromohydroxylation[3] of 3,4-dihydro-2*H*-pyran (DHP) (Scheme 1) and 2,3-dihydrofuran (DHF) (Scheme 2).
2. *Candida antarctica* lipase B (CALB)-catalyzed kinetic resolution[4,5] of enantiomers of α-bromo-ω-hydroxyaldehydes[6,7] upon acetylation or deacetylation followed by chromatography.
3. Treatment of optically resolved compounds (bromohemiacetals or their acetates) with base in alcohol.[8]

The bromination[9] of DHP resulted in *rac*-1*trans* in high yield (≥90%), while DHF afforded *rac*-3*trans* in moderate yield (~50%). The products[10] are formed as *trans* isomers according to the mechanism of the reaction. Actually, we observed (by NMR, HPLC) the equilibria between anomers characteristic of a certain structure. It is probable that all the isomeric forms could exist (including open-chain forms, despite our failure to detect those experimentally) pictured as Set C (Scheme 1) and Set F (Scheme 2) in solution.

The single anomers obtained by HPLC separation were observed to undergo a rapid re-establishment of the initial equilibrium under the conditions used (rt; then evaporation at 35°C under reduced pressure). The dynamic equilibration observed explains the high discrimination of the *cis* anomer along with a high total yield of the products in both the chemical and enzymatic reactions performed. No *cis* isomer was detected in the product of the lipase-catalyzed acetylation (conv.: ≥98%) of bromohemiacetals: Set C→Set D (Scheme 1). The optically pure material (Set A; (2*R*)-2*trans* of Set D) was treated with LiOH in methanol affording *trans*-glycoside (2*S*)-5*trans* upon the double S_N2 process (Scheme 1) in >90% yield and >98% iso-

Keywords: lipase; deoxysugar alkyl glycoside; decyclization of hemiacetal by lipase; α-bromo-ω-hydroxyaldehyde.

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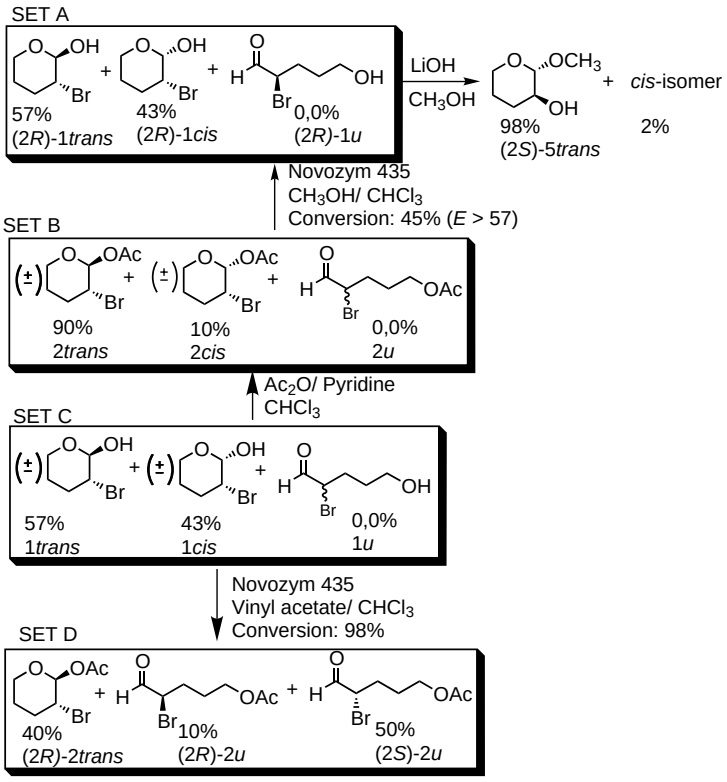
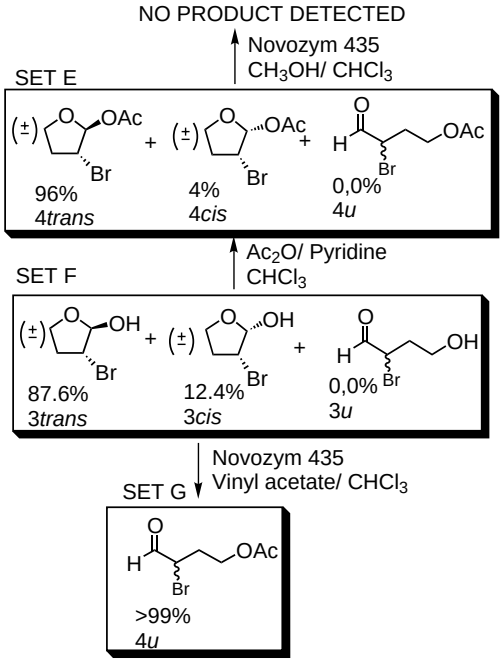


Scheme 1. Synthesis of deoxysugar methyl glycoside (2*S*)-5*trans*. Chemical and lipase-catalyzed acetylation (deacetylation) of *rac*-2-bromo-5-hydroxypentanal.



Scheme 2. Synthesis and chemical and lipase-catalyzed acetylation of *rac*-2-bromo-4-hydroxybutanal.

meric purity. In this reaction only the *trans* hemiacetal anomer is reactive. The absolute configuration of (2*S*)-5*trans* was assigned by the results of the studies of differential shielding effects observed in the NMR spectra of THP–mandelate diastereomers[11] confirming also the absolute configuration of (2*R*)-1*trans*.

The chemical acetylation of bromohemiacetals (Set C→Set B; Set F→Set E) resulted in isomeric stable acetates of a nearly 3–4-fold diminished content of the *cis* anomer.

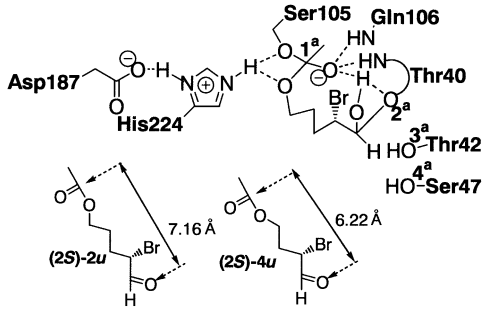
The open-chain α-bromo-ω-hydroxyaldehyde (2*S*)-1*u* together with some amount of (2*R*)-1*u*; (Scheme 1) both of minute thermodynamic probability (Table 1) were stabilized by CALB (Scheme 3) followed by trapping of these isomeric forms by acetylation[12] occurring during the prolonged incubation (60 h; conversion rate $\geq$98%), while the enantioselective acetylation (E$\geq$67) of cyclic *trans*-hemiacetal (2*R*)-1*trans* was found to be rapid (3 h; conversion rate=45%) under the same conditions. In the case of C_4–aldehyde hemiacetals none of the isomeric cyclic acetates (of Set E) was formed nor cleaved upon CALB-catalytic reactions[13] in our hands. The product obtained from the lipase-catalyzed acetylation in ~98% yield was an almost racemic mixture of open-chain acetates (Set G).

QC calculations were performed in an attempt to explain the anomer ratio dynamics as well as the logic of acetylation of open-chain hydroxyaldehydes.

For all hemiacetals, open-chain aldehydes and the corresponding acetates (Table 1) an attempt to find conformers with minimum energy was made with the help of Tinker's[14] SCAN program.

Table 1. Thermodynamic characteristics calculated for the most probable conformers of isomeric forms of α-bromo-ω-hydroxyaldehydes and the corresponding acetates. Thermodynamic and experimentally (NMR) determined distribution of isomeric forms for products of chemical synthesis

Set	Compound	Heat of formation (kcal/mol)	Difference of heat of formation between conformers of all isomers	Percentage of an isomeric form	
				Calculated	Experimental
C	1*trans*	−96.19	0.00	65.9	57
	1*cis*	−95.66	0.53	34.1	43
	1*u*	−86.73	9.46	0.0	0.0
F	3*trans*	−89.82	0.00	89.3	87.5
	3*cis*	−88.38	1.44	10.7	12.5
	3*u*	−81.23	8.59	0.0	0.0
B	2*trans*	−137.34	0.00	26.6	90
	2*cis*	−136.80	0.54	10.6	10
	2*u*	−136.37	0.97	62.8	0.0
E	4*trans*	−131.38	0.00	47.7	≥96
	4*cis*	−128.99	2.39	1.4	≤4
	4*u*	−130.26	1.12	50.9	0.0



Scheme 3. Probable tetrahedral intermediate of CALB-catalytic ω-acetylation of (2*S*)-2-bromo-5-hydroxypentanal. [a]: (1) C_1 of acetyl docked to CALB; (2) oxygen atom (OA) of the hydroxyl group (HG) of Thr40; (3) OA of the HG of Thr42; (4) OA of the HG of Ser47. The distances between labeled atoms: (1 and 2) 3.9 Å; (1 and 3) 7.65 Å; (1 and 4) 8.00 Å. The distances measured are based on the crystal structure of CALB structure 1 TIB[19] acquired from Protein Data Bank.[20]

This program performs a general conformational search for the entire potential energy surface via a basin hopping algorithm,[15] and it also minimizes each conformer after its generation. The force field chosen, MM3,[16] is known to be quite successful in reproducing molecular IR spectra.

All the conformers generated were additionally minimized using the Alchemy 2000[17] program and the same MM3 method therein. This was performed because the MM3 force field within the latter program has improvements upon Tinker's version, viz. the anomeric and Bohlmann correction terms[18] have been implemented.

The percentage of isomeric forms in the equilibrium of α-bromo-ω-hydroxyaldehydes calculated for Set C and Set F is in approximate agreement with experimental results.

The chemical acetylation of α-bromo-ω-hydroxyaldehydes as an isomeric equilibrium mixture of cyclic hemiacetals afforded isomeric stable acetates of a changed ratio. The content of *cis* acetates, 2*cis* and 4*cis*, in the products (Set B and Set E, respectively) was found to correspond to the thermodynamical distribution of isomeric acetates whereas instead of open-chain acetates expected by calculation only *trans* acetates were formed.

The cyclic *trans* hemiacetal (2*R*)-1*trans* favored by lipase is acetylated by CALB rapidly, but even this aldehyde enantiomer was partially acetylated as an open-chain form, probably because of the presence of the *cis* anomer not favored by CALB, thus allowing coordination of the aldehyde molecule for transhemiacetalization/decyclization. Another aldehyde enantiomer not favored by CALB, in both cyclic forms (hemiacetals), was almost totally acetylated as an open-chain isomer.

Conclusions

1. The observed thermodynamic control of *cis* acetate formation during the chemical acetylation suggests that the activated intermediate complex of the hemiacetal acetylation has to proceed via an open- or quasi-open-chain state allowing realization of intramolecular interactions characteristic of acetate molecules. By using chemical methods no acetylation of the ω-OH group of the open-chain isomer of any hydroxyaldehyde was observed.
2. During the enzymatic acetylation of the C_5–aldehyde (Set C) the 2*S*-enantiomer was stabilized by the lipase as the open-chain isomer followed by trapping by acetylation, while the 2*R*-enantiomer afforded preferentially the cyclic *trans* acetate (2*R*)-2*trans*. CALB-catalyzed acetylation of C_4–aldehyde (Set F) resulted in an almost racemic mixture of open-chain acetates (Set G).

3. The asymmetric chemoenzymatic synthesis of *trans*-(2*S*,3*S*)-2-methoxy-tetrahydropyran-3-ol was elaborated.
4. Differences in the heat of formation between conformers of all isomers of 2-bromo-4-hydroxybutanal and 2-bromo-5-hydroxypentanal allow the estimation of the hemiacetal decyclization energies: 7.15–8.59 kcal/mol for the C_4–aldehyde and 8.93–9.46 kcal/mol for the C_5–aldehyde. These values correspond to the total energies of 4–5 hydrogen bonds seeming improbable even with the help of the 'oxy-anion hole' of CALB[21] which, besides, has to be engaged in a simultaneous stabilization of the tetrahedral intermediate of acetylation of the ω-hydroxyl group (involving Ser105).
5. Based on the results of measurements of molecular geometry in the active site of CALB, we expect the stabilization of the open-chain form of the hydroxyaldehyde molecule to occur covalently upon transhemiacetalization with the hydroxyl group of Thr40. (However, this process could be evoked by initial influence of the 'oxy-anion hole' of lipase on the hydroxyaldehyde.)

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9. **Experimental**. **Chemical acetylation**. Chloroform was added to a racemic mixture (1 equiv.) of bromohemiacetals resulting in a 0.4 M solution. Acetic anhydride (1.5 equiv.) and pyridine (5.4 equiv.) were added at rt with stirring. After stirring overnight the reaction mixture was washed with $NaHCO_3$ and brine and dried over Na_2SO_4, filtered and evaporated. Crude products were investigated by using NMR spectroscopy (conversion rate stated: >98%; the isomer ratios are presented in Table 1). Samples were purified over silica (15% EtOAc in hexane). Total yield of products was about 50%. Reaction time: 18 h (Set C→Set B; Set F→Set E). **Lipase-catalyzed acetylation**. Substrate (100 mg) was added to a mixture of chloroform and vinyl acetate (3:1) resulting in a 0.138 M solution. Novozym 435 (50 mg) was added per 0.552 mmol of substrate at rt. The solution was stored without stirring at rt. The reaction was terminated at the conversion rate according to the goal of the experiment by filtering off the enzyme, the solution was evaporated and the residue was studied using NMR spectroscopy, further purified over silica and characterized. **Lipase-catalyzed deacetylation**. Substrate (100 mg, 1 equiv.) and methanol (44 equiv.) were added to chloroform resulting in a 0.138 M solution. Novozym 435 (76 mg) was added per 1 mmol of substrate without stirring at rt. After reaching the conversion rate of 45% the solution was filtered, evaporated (studied by NMR) and purified over silica. Reaction time: 6 h (Set B→Set A; Set E→no product was detected after 60 h of incubation). **Synthesis of deoxy-sugar methyl glycoside**. 3-Bromo-tetrahydro-pyran-2-ol (1 equiv., 4.42 mmol) and 2.5 equiv. LiOH were added to methanol (16 ml) at rt. After stirring for 1 h at rt the solution was evaporated and purified over silica (1/1 EtOAc/hexane).
10. **Characterization of compounds**. **(2*R*)-1*trans*+(2*R*)-1*cis* (57/43)**: TLC R_f=0.4 (1/9 acetone/C_6H_6); $[\alpha]_{546}^{20}$ −4.76 (*c* 0.01; C_6H_6); **(2*R*)-1*trans***: ^{13}C and ^1H NMR C_1 97.00 (4.84), C_2 50.93 (3.87), C_3 31.86 (1.93/2.40), C_4 25.11 (1.59/1.80), C_5 64.46 (3.58/4.04); $^3J_{H1,H2}$=6.4 Hz; **(2*R*)-1*cis***: ^{13}C and ^1H NMR C_1 92.55 (4.76), C_2 53.76 (4.24), C_3 29.67 (2.09/2.32), C_4 23.04 (1.58/1.88), C_5 63.01 (3.56/4.04); $^3J_{H1,H2}$=1.8 Hz; **(2*S*)-5*trans***: TLC R_f=0.35 (5/5 EtOAc/hexane); $[\alpha]_{546}^{20}$ +46 (*c* 0.005; C_6H_6); ^{13}C and ^1H NMR C_1 102.80 (4.23), C_2 67.85 (3.43), C_3 27.45 (1.50/1.90), C_4 22.08 (1.39/1.69), C_5 62.86 (3.40/3.75), C_{CH3} 55.48 (3.35); $^3J_{H1,H2}$=5.1 Hz; **3*trans***: TLC R_f=0.4 (4/6 EtOAc/hexane); ^{13}C and ^1H NMR C_1 103.29 (5.58), C_2 50.82 (4.22), C_3 33.27 (2.22/2.68), C_4 66.95 (4.15/4.16); $^3J_{H1,H2}$ <1 Hz; **3*cis***: TLC R_f=0.4 (4/6 EtOAc/hexane); ^{13}C and ^1H NMR C_1 96.62 (5.26), C_2 50.33 (4.26), C_3 33.20 (2.38/2.52), C_4 66.45 (3.89/4.16); $^3J_{H1,H2}$=3.6 Hz. **(2*R*)-2*trans***: TLC R_f=0.5 (3/7 EtOAc/hexane); $[\alpha]_{546}^{20}$ −74.8 (*c* 0.01; C_6H_6); ^{13}C and ^1H NMR C_1 94.22 (5.85), C_2 47.09 (3.99), C_3 30.18 (2.01/2.40), C_4 23.21 (1.60/1.95), C_5 64.30 (3.72/3.96), C_1' 169.03, C_2' 20.80 (2.11); $^3J_{H1,H2}$=5.2 Hz; **2*cis***: TLC R_f=0.5 (3/7 EtOAc/hexane); ^{13}C and ^1H NMR C_1 90.78 (6.06), C_2 46.89 (4.16), C_3 29.01 (2.15/2.25), C_4 26.10 (1.80), C_5 61.02 (3.68/3.85), C_1' 169.03, C_2' 20.72 (2.16); $^3J_{H1,H2}$=2.8 Hz; **(2*S*)-2*u***: TLC R_f=0.54 (3/7 EtOAc/hexane); $[\alpha]_{546}^{20}$ −67.4 (*c* 0.02; C_6H_6); ^{13}C and ^1H NMR C_1 192.26 (9.45), C_2 54.60 (4.27), C_3 28.05 (1.75/2.10), C_4 26.10 (1.75/2.10), C_5 63.21 (4.10), C_1' 170.64, C_2' 20.04 (2.05); **4*trans***: TLC R_f=0.5 (3/7 EtOAc/hexane); ^{13}C and ^1H NMR C_1 102.48 (6.28), C_2 48.82 (4.27), C_3 33.01 (2.24/2.57), C_4 68.06 (4.17/4.18), C_1' 169.37, C_2' 20.89 (1.99); $^3J_{H1,H2}$ <1 Hz; **4*cis***: TLC R_f=0.5 (3/7 EtOAc/hexane); ^{13}C NMR C_1 95.30, C_2 43.76, C_3 32.20, C_4 67.36; $^3J_{H1,H2}$=4.1 Hz. **4*u***: TLC R_f=0.37 (3/7

EtOAc/hexane); ^{13}C and ^{1}H NMR C_1 191.75 (9.48), C_2 51.46 (4.40), C_3 30.73 (2.18/2.45), C_4 61.07 (4.20/4.25), C'_1 170.60, C'_2 20.72 (2.05).

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