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Efficient Synthesis of Either Enantiomer of Ethyl 5-Hydroxyhept-6-enoate

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Dedicated to Prof. Dr. M.-R. Kula on the occasion of her 70th birthday.

Abstract: The application of alcohol dehydrogenases as a key-step for the synthesis of the title compound is reported. 5-Hydroxyhept-6-enoates are versatile intermediates, e.g., for the synthesis of a variety of arachidonic acid metabolites.

Keywords: asymmetric catalysis; chemoenzymatic synthesis; enantioselectivity; enzyme catalysis; oxidoreductases; reduction

5-Hydroxyhept-6-enoates (**1**) have been used as key intermediates for a variety of physiologically active compounds (Figure 1). Primary targets were metabolites of the arachidonic acid pathway, e.g., prostaglandins,[1] leukotrienes,[2] or isoprostanes,[3] but other natural products such as atractyligenin[4] or terpenes[5] were approached using alcohol **1**, too. Although racemic mixtures were introduced in some cases, enantioselective routes were also developed. While the CBS reduction (Corey–Bakshi–Shibata)[6] failed to give high yield and selectivity[4] (*vide infra*) when reducing the corresponding ketone, ω-stannylated or ω-silylated derivatives were successfully used, albeit additional steps were essential.[4] An alternative 11 step sequence starting from D-arabinose was recently disclosed.[3] Here, we describe a short synthesis of either enantiomer of **1** (with *ee* >99%) utilizing alcohol dehydrogenases (ADHs)[7] for the key enantioselective reducing step.

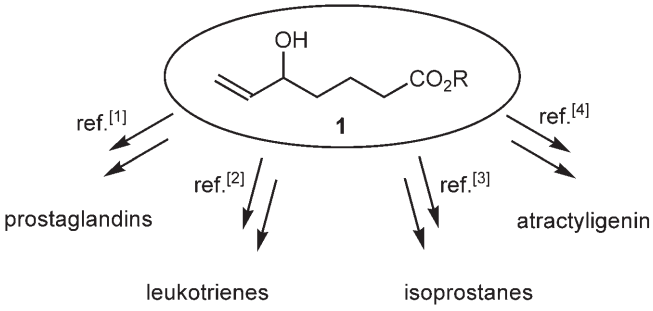
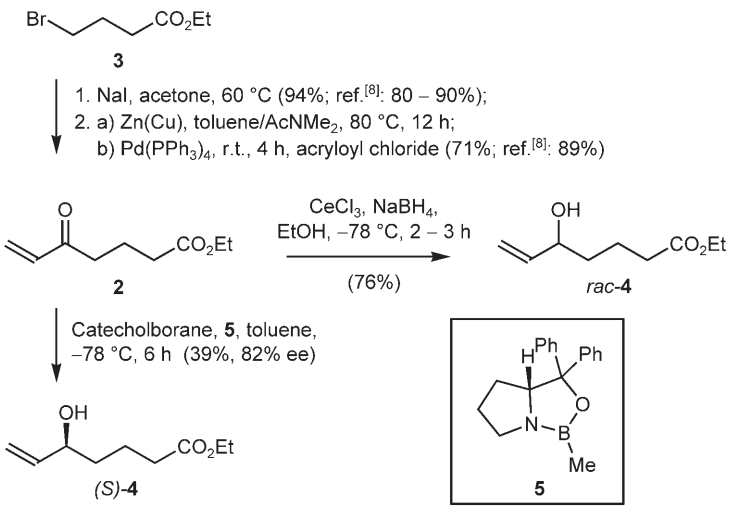


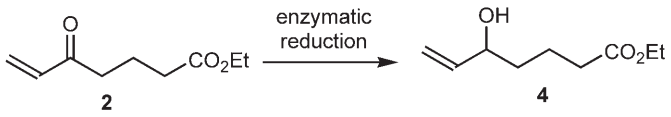
Figure 1.

First, we established the analytical basis for the enzymatic transformation. The synthesis of the starting material, ketone **2**, was conveniently achieved *via* two well established steps from the commercially available bromide **3** (Scheme 1).[8] Reduction under Luche conditions[9] with $NaBH_4/CeCl_3$ led to the racemic mixture of the desired alcohol **4**. In order to assign the configuration, an enantioselective CBS reduction with catecholborane in the presence of oxazaborolidine **5** was performed. The (*S*)-enantiomer was obtained in low yield and moderate selectivity (39% yield, 82% *ee*), as was previously found for the corresponding methyl ester under similar conditions.[4] Gas chromatographic separation of the enantiomers was achieved using Lipodex G as chiral stationary phase.

For the enzymatic reduction eight ADHs were tested using two different buffer systems. First, an optical enzyme activity test was performed utilizing the decrease of extinction at 340 nm for NAD(P)H. Two enzymes stood out right from the beginning (Scheme 2, entries 2, 3, 10, 11): ADH-LB (from *Lactobacillus brevis*) and ADH-T (from *Thermoanaerobacter* species recombinant in *E. coli*). The results were confirmed when repeating the biotransformation in an analytical scale. High conversion was observed especially with these enzymes (ADH-T: 95% and 91%, respectively; ADH-LB: 94% in KP_i buffer), moreover ADH-RS1 (from *Rhodococcus* species) gave also good results in KP_i buffer (85%). The enantioselectivity – as determined by GLC – was high, in some cases (entries 2 and 5) >99% *ee*. It is interesting to note that (*R*)-**4** is preferentially formed in all cases, but ADH-LB gave the (*S*)-enantiomer. In view of the complimentary high selectivity and the observed conversion we decided to pursue the synthesis



Scheme 1. Synthesis of reference compounds.

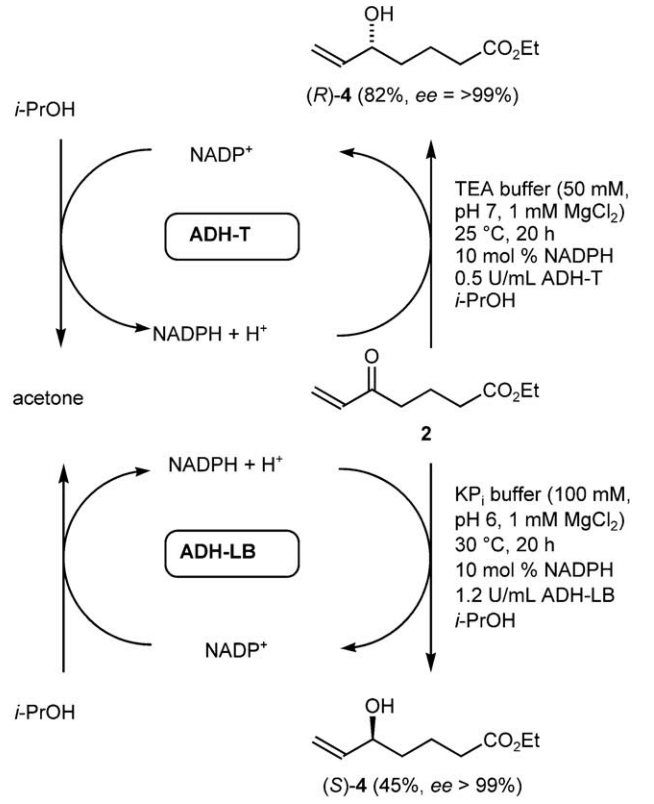


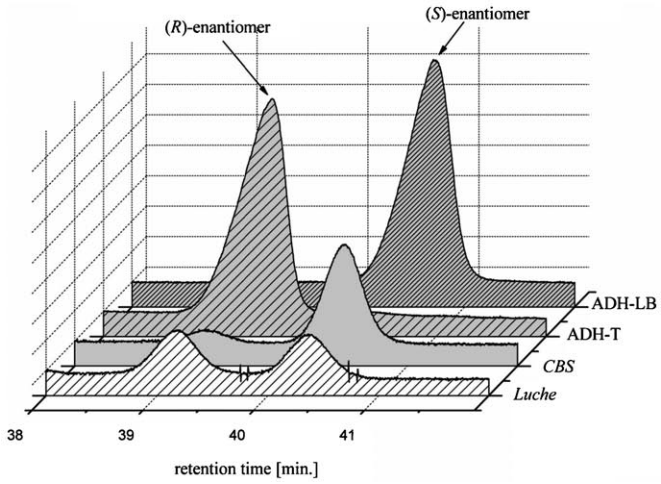
Entry	Enzyme	Buffer	U/mL	Cofactor	Conversion	*ee*	Product
1	ADH-RS1	TEA	45.6	NADH	9%	83%	(*R*)-**4**
2	ADH-LB	TEA	161	NADPH	37%	>99%	(*S*)-**4**
3	**ADH-T**	**TEA**	**320**	**NADPH**	**95%**	**98%**	**(*R*)-4**
4	ADH-RS2	TEA	21	NADH	20%	90%	(*R*)-**4**
5	ADH-CP	TEA	0.2	NADH	17%	>99%	(*R*)-**4**
6	ADH-CDX010	TEA	0.8	NADH	-	-	-
7	ADH-PF	TEA	0.3	NADPH	-	-	-
8	ADH-CDX013	TEA	1.0	NADPH	-	-	-
9	ADH-RS1	KP_i	48	NADH	85%	95%	(*R*)-**4**
10	**ADH-LB**	**KP_i**	**161**	**NADPH**	**94%**	**95%**	**(*S*)-4**
11	ADH-T	KP_i	320	NADPH	91%	57%	(*R*)-**4**
12	ADH-RS2	KP_i	22.5	NADH	7%	95%	(*R*)-**4**
13	ADH-CP	KP_i	0.4	NADH	15%	95%	(*R*)-**4**
14	ADH-CDX010	KP_i	1.6	NADH	-	-	-
15	ADH-PF	KP_i	0.3	NADPH	-	-	-
16	ADH-CDX013	KP_i	1.0	NADPH	-	-	-

Scheme 2. Alcohol dehydrogenase activity tests (U/mL) were performed by photometric measurement of the NAD(P)H consumption (TEA: 50 mM triethanolamine, pH 7; KP_i: 100 mM K_2HPO_4/KH_2PO_4, pH 6). All tested enzymes are commercially available as solutions (50% in glycerol) from Julich Chiral Solutions; the enzymes were not further purified.

on a semi-preparative scale with ADH-T (in TEA buffer: 98% *ee*) and ADH-LB (in KP_i buffer: 95% *ee*) (Scheme 3). Cofactor recycling was achieved by adding 1.5 vol% 2-propanol. In both cases the *ee* could be enhanced (*ee* >99%); the yield of isolated allylic alcohol (*R*)-**4** was 82%. The synthesis of the (*S*)-enantiomer (*S*)-**4** will require some more optimization (yield: 45%). However, when comparing the results with previous syntheses, the chemoenzymatic approach would seem superior.

In case of the ADH-T, a side-product was observed during the screening phase by GLC to an extent that we never detected for any of the other enzymes. The retention time [17.4 min; Lipodex G, H_2 (0.6 bar), 90 °C iso] generally supported the suspicion that lactonization occurred. We proved it by independent





Scheme 3. Enzymatic reduction on a preparative scale: synthesis of (*R*)- and (*S*)-**4** and detail of GLC trace {GLC conditions: Lipodex G, H_2 (0.6 bar), 90 °C iso, t_R [(*R*)-**4**] = 39.4 min, t_R [(*S*)-**4**] = 40.4 min}.

chemical synthesis of both, racemic and enantiomerically pure lactone *rac*-/(*R*)-**6** (Scheme 4). When repeating the enzymatic transformation on a preparative scale, the enantiomerically pure lactone could be isolated in up to 10% yield. Experiments to explain the formation of the vinyllactone (*R*)-**6** are currently in progress.

Summing up, we report a 3-step chemoenzymatic synthesis of allyl alcohol (*R*)-**6** (*ee* >99%) in 55% overall yield. Key to the success was an enzymatic reduction that proved to be superior to conventional synthetic reducing reagents. Further investigations to utilize the observation and to further increase the turnover number for NAD(P)H recycling are in progress.

Experimental Section

Synthesis of (*R*)-Ethyl 5-Hydroxyhept-6-enoate [(*R*)-4]

Ketone **2** (170 mg, 1.00 mmol) in 1.5 mL 2-propanol, NADPH (83.3 mg, 0.10 mmol) and 31 μL ADH-T solution (~50% in glycerol, as supplied by Julich Chiral Solutions) were added to 100 mL TEA buffer (50 mM, pH 7, + 1 mM $MgCl_2$). The reaction mixture was stirred for 20 h (95% conversion as judged by GLC) at room temperature. After filtration the aqueous solution was extracted with dichloromethane (3×), the organic layer dried over $MgSO_4$, filtered, and the solvents removed under reduced pressure. The crude product was subjected to flash column chromatography (petroleum ether:ethyl acetate, 70:30; R_f: 0.26) to afford product (*R*)-**4** as a colorless oil; yield: 141 mg (818 μmol, 82%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 1.26 (t, $^3J_{2',1'}$ = 7.1 Hz, 3H, 2′-H), 1.57 (m_c, 2H, 4-H), 1.67–1.79 (m, 3H, 3-H, -OH), 2.34 (t, $^3J_{2,3}$ = 7.0 Hz, 2H, 2-H), 4.11 (m_c, 1H, 5-H), 4.13 (q, $^3J_{1',2'}$ = 7.1 Hz, 2H, 1′-H), 5.12 (ddd, $^3J_{7a,6}$ = 10.4 Hz, $^2J_{7a,7b}$ = 1.4 Hz, $^4J_{7a,5}$ = 1.2 Hz, 1H, 7-H_a), 5.24 (ddd, $^3J_{7b,6}$ = 17.4 Hz, $^2J_{7b,7a}$ = 1.4 Hz, $^4J_{7b,5}$ = 1.4 Hz, 1H, 7-H_b), 5.87 (ddd, $^3J_{6,7b}$ = 17.4 Hz, $^3J_{6,7a}$ = 10.4 Hz, $^3J_{6,5}$ = 6.2 Hz, 1H, 6-H); ^{13}C NMR ($CDCl_3$, 150 MHz): δ = 14.3 (C-2′), 20.8 (C-3), 34.1 (C-2), 36.3 (C-4), 60.4 (C-1′), 72.7 (C-5), 114.9 (C-7), 140.9 (C-6), 173.6 (*C*=O); $[\alpha]_D^{20}$: −71.2 (*c* 1.10, $CHCl_3$); >99% *ee*, as determined by GLC: *Lipodex G,* H_2 (0.6 bar), 90 °C iso, t_R [(*R*)-**4**] = 39.4 min.

Scheme 4. Formation of vinyllactone **6**.

Acknowledgements

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