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Bioreductive synthesis of perfluorinated chiral alcohols

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Abstract—Perfluorinated chiral alcohols are interesting building blocks for pharmaceuticals and agrochemicals. Different chiral (*R*)- and (*S*)-configured perfluorinated alcohols were produced by asymmetric reduction of the corresponding ketones. Commercially available alcohol dehydrogenases were used as catalyst in combination with different cofactor regenerating systems. High selectivities of >99% were observed in most cases. The results also demonstrate the influence of the CF_3 group on reactivity and enantioselectivity of alcohol dehydrogenases.

Fluorinated compounds are important building blocks for biologically active compounds, such as pharmaceuticals and agrochemicals, due to the unique properties of the fluorine substituent.[1] Because of the need of chiral molecules in new drug developments, the preparation of chiral fluorinated building blocks is of particular interest. As example ethyl (*R*)-3-hydroxy-4,4,4-trifluorobutyrate was used as an intermediate for the antidepressant Befloxatone.[2] Methods for the stereoselective synthesis of chiral fluorinated alcohols have been described.[3] Especially, asymmetric reduction of prochiral fluorinated ketones is an efficient approach. As reagents, chiral boranes,[3] and chiral aluminium hydride reagents,[4] or transition metal-catalyzed hydrogenation[5] were used. Also bioreductive techniques were examined. Whole cells such as baker's yeast were applied for the reduction of a range of fluorinated ketones.[6] Still, most of these methods yield the products with enantiomeric excess less than 99%. Isolated alcohol dehydrogenases (ADH) are usually more selective than whole cell systems such as baker's yeast, which usually contains different enzymes with opposite stereoselectivity. We recently produced different chiral fluorinated alcohols as building blocks for early stage developments of new pharmaceuticals. These products were synthesized by asymmetric reduction of perfluorinated ketones with both (*R*)- and (*S*)-selective commercially available alcohol dehydrogenases. The following alcohol dehydrogenases were used as isolated enzymes: alcohol dehydrogenase from *Lactobacillus brevis* (ADH LB),[7] alcohol dehydrogenase from *Thermoanaerobacter* spec. (ADH T),[8] and two alcohol dehydrogenases from *Rhodococcus* species (ADH RS1 and RS2).[9] ADH LB is the only (*R*)-selective enzyme among these biocatalysts.

Initially, the activity of these ADHs for the conversion of the perfluorinated aliphatic and aromatic ketones as well as a β-ketoester (**1**–**5**) was measured using a photometrical assay (Table 1).[10] The results revealed good activities of ADH LB and T for the conversion of all tested perfluorinated ketones. Only for the sterically demanding ketone, 1-(9-anthryl)-2,2,2-trifluoroethanone (**4**), no activity was found. Low activity was found for ADH RS2. ADH RS1 showed only activity for the aliphatic ketones **1**, **2** and **5**. Comparison of the activities with the nonfluorinated ketone shows that replacement of a CH_3 by a CF_3 group leads to significantly decreased activity. The effect is very significant for ADH RS1 (entries 5 and 6). This indicates the larger steric demand of the trifluoromethyl group. Interestingly, higher activities were found for 3,3,4,4,4-pentafluorobutanone (**2**) compared to 1,1,1-trifluoroacetone (**1**) and 2-butanone, indicating higher reactivity of the carbonyl group.

After measuring the photometrical activity based on NAD(P)H consumption, small scale conversions were made to examine yield and selectivity for the ADH-catalyzed reduction of the perfluorinated ketones **1**–**5**. The hydride donating cofactors NADH or NADPH were regenerated by different methods.

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Table 1. Results of photometrical activity of different isolated ADHs for the reduction of perfluorinated ketones

Entry	R_1	R_f	Relative activity of ADHs in %			
			ADH LB[a]	ADH T[b]	ADH RS1[c]	ADH RS2[d]
1	CH_3	CF_3	61	26	1.4	10
2	CH_3	CH_3	73	100	10	2.8
3	CH_3	C_2F_5	133	65	30	n.d.
4	CH_3	C_2H_5	94	72	10	6.5
5	CH_2–CO_2Et	CF_3	6.75	10	2	6.8
6	CH_2–CO_2Et	CH_3	148	21	296	89
7	Ph	CF_3	101	28	<1	4.6
8	Ph	CH_3	100	52	15	23
9	9-Anthryl	CF_3	1.25	1.8	<1	6.9

[a] Characteristic activity is 1000 U/mL with the standard substrate acetophenone.
[b] Characteristic activity is 1000 U/mL with the standard substrate acetone.
[c] Characteristic activity is 100 U/mL with the standard substrate *p*-chloro acetophenone.
[d] Characteristic activity is 59 U/mL with the standard substrate chloro acetone.

Since ADH LB and ADH T tolerate high concentrations of 2-propanol, the cofactor can be regenerated by addition of an excess of this alcohol (10–40 vol %). In a typical experiment, 2 mmol (200 mM) of the corresponding ketones **1**–**5** and 0.001 mmol (0.1 mM) of $NADP^+$ were dissolved in a mixture of 6–8 mL Tris/HCl-buffer (pH 7.0). 2-Propanol (2–4 mL) was added so that a reaction volume of 10 mL is obtained. The reaction was started by the addition of a crude cell-free extract of the corresponding enzyme (1 U/mL based on results of the photometrical assay). The reaction was stirred for 24 h at 30 °C. Alternatively, a second enzymatic system such as glucose dehydrogenase (GDH) from *Bacillus subtilis* (GDH BS) or *Bacillus megaterium* (GDH BM) and glucose was used as cofactor regeneration system. Formate dehydrogenase from *Candida boidinii* (FDH CB) and sodium formate was used in conversions with ADH RS1. For these experiments, GDH BM/BS (1 U/mL) or FDH CB (1 U/mL) was added to the reaction mixture. In case of GDH, 3.5 mmol (350 mM) glucose and 3.0 mmol (300 mM) $CaCO_3$ were added, for FDH 5.0 mmol (500 mM) sodium formate was added. Results of the small conversions are summarized in Table 2 (Scheme 1).

Both aliphatic ketones, 1,1,1-trifluoroacetone (**1**) and 3,3,4,4,4-pentafluorobutanone (**2**), were converted by all of the tested ADHs. Nevertheless, enantioselectivity of (*S*)-selective ADHs was only 80–93% for **1**. Using ADH LB as catalyst, the corresponding (*R*)-configured alcohol was obtained with ee 94% and 98% dependent on the cofactor regeneration.[11] If the perfluorinated group is increased from CF_3 to C_2F_5, ADH LB and ADH RS1 would give the corresponding alcohol with high ee of >99%. Only low selectivities were found for the reduction of the unfluorinated 2-butanone: ADH LB 97% ee (2-propanol), ADH T 34% ee (2-propanol), ADH RS1 62% ee (FDH CB).[12] The larger steric demand of the trifluoromethyl group decreases enzymatic activity and increases the selectivity of the ADHs. Enzymatic activity for the conversion of 1,1,1-trifluoroacetophenone (**3**) was only found for ADH LB, T and RS2. ADH LB and T gave both the (*S*)-enantiomer, with very high selectivities of ee >99%.[13] From the results for the conversion of unfluorinated acetophenone, it was expected that ADH T and RS2 would yield the (*R*)-enantiomer. Models were developed to explain the unexpected stereoselectivity for the reduction of **3** with aluminium reagents.[4b,c] From these models, it can be concluded that the transition state for the ADH T-catalyzed reaction is influenced by the steric demand of the CF_3 group leading to an inverse selectivity. Interestingly, this does not influence the selectivity of ADH LB. No conversion of 1-(9-anthryl)-2,2,2-trifluoroethanone (**4**) was observed for all enzymes. Steric hindrance is expected to be problematic. Finally, ethyl 4,4,4-trifluoro-3-oxobutyrate (**5**) was reacted with different ADHs. All ADHs showed very high enantioselectivity of >99%. ADH LB yielded the corresponding (*S*)-enantiomer as determined by optical rotation.[14] All other enzymes gave the corresponding (*R*)-isomer. Reactions with ADH LB or T and 40% 2-propanol gave only low conversions after 24 h. Full conversion was observed after longer reaction times.

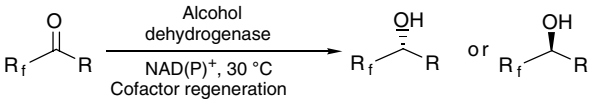
The results were used to produce (*S*)- and (*R*)-1,1,1-trifluoro-2-propanol ((***S***)- and (***R***)-**6**) on a 100 g scale for an early stage pharmaceutical development project. Both enantiomers were produced before according to the literature by asymmetric reduction of 1,1,1-trifluoroacetone,[3c,6b] or by resolution reactions.[15] Unfortunately, cofactor regeneration with 2-propanol was not possible because of the similar boiling point. (*R*)-1,1,1-Trifluoro-2-propanol ((***R***)-**6**) was produced using ADH LB and GDH BM for cofactor regeneration.[16] The reaction was performed at 15 °C, which led to a slightly improved selectivity from 94% (Table 2, entry 2) to 97%. (*S*)-1,1,1-Trifluoro-2-propanol ((***S***)-**6**) was produced using ADH RS1 and FDH CB for cofactor regeneration. The reaction was also performed at 15 °C, which resulted in an ee of 93% as observed in the small scale preparations (Table 2, entry 1).[17]

The results demonstrate the broad applicability of alcohol dehydrogenases for the production of perfluorinated chiral alcohols by asymmetric reduction. It was also shown that the introduction of a perfluorinated residue influences the activity and selectivity of analogous reactions.

Table 2. Results of the asymmetric reduction of perfluorinated ketones with different isolated recombinant alcohol dehydrogenases

Entry	R_1	R_f	ADH LB			ADH T			ADH RS1			ADH RS2		
			NADPH regeneration	Conversion[a] %	ee[b] %	NADPH regeneration	Conversion[a] %	ee[b] %	NADH regeneration	Conversion[a] %	ee[b] %	NADH regeneration	Conversion[a] %	ee[b] %
1[c]	CH_3	CF_3	20% ^{i}PrOH	n.d.	98 (*R*)	20% ^{i}PrOH	n.d.	83 (*S*)	FDH CB $NaHCO_2$	100	93 (*S*)	GDH BM Glucose	90	90
2[c]	CH_3	CF_3	GDH BM Glucose	100	94 (*R*)	GDH BM Glucose	8	80 (*S*)						
3[c]	CH_3	C_2F_5	GDH BM Glucose	53	>99 (*R*)				FDH CB $NaHCO_2$	100	>99 (*S*)		n.t.	
4[d]	Ph	CF_3	30% ^{i}PrOH	87	>99 (*S*)	30% ^{i}PrOH	89	>99 (*S*)		n.t.		GDH BM Glucose	12	98 (*R*)
5[d]	Ph	CF_3	GDH BS Glucose	64	99 (*S*)	GDH BS Glucose	55	>99 (*S*)		n.t.				
6[c]	9-Anthryl	CF_3	30% ^{i}PrOH	0	n.d.	30% ^{i}PrOH	0	n.d.		n.t.		GDH BM glucose	0	n.d.
7[c]	9-Anthryl	CF_3	GDH BS Glucose	0	n.d.	GDH BS Glucose	0	n.d.		n.t.				
8[e]	CH_2CO_2Et	CF_3	40% ^{i}PrOH	23	>99 (*S*)	40% ^{i}PrOH	4	>99 (*R*)	GDH BS Glucose	90[f]	>99 (*R*)	GDHBM Glucose	23	>99 (*R*)

n.t. – not tested.
n.d. – not determined.
[a] Conversion after 24 h, determined by GC.
[b] Determined by chiral GC.
[c] Concentration of the ketone, 200 mM.
[d] Concentration of the ketone, 100 mM.
[e] Concentration of the ketone, 250 mM.
[f] Concentration of the ketone, 50 mM.



1, R = CH_3, R_f = CF_3
2, R = CH_3, R_f = C_2F_5
3, R = Ph, R_f = CF_3
4, R = 9-Anthryl, R_f = CF_3
5, R = CH_2CO_2Et, R_f = CF3

Scheme 1. Reduction of perfluorinated ketones with alcohol dehydrogenases.

References and notes

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10. The enzymatic assay for the activity measurement of the alcohol dehydrogenases was done as follows: 20 μL of a solution of NAD(P)H (10 mM in potassium phosphate buffer, pH 7.0) was added to a solution of 970 μL of 50 mM potassium phosphate buffer, pH 7.0, and 10 mM of the corresponding ketone at 30 °C. The reaction was started by adding 10 μL of the corresponding enzyme solution (diluted to 0.5–1.5 U/mL). The cuvettes were thermostated and the activity was measured at 30 °C. The consumption of NAD(P)H was measured by UV photometry at 340 nm. The activities were calculated as enzyme units (U, i.e., μmol/min) by using a molar extinction coefficient of 6220 M^{-1} cm^{-1}.
11. Absolute configuration was determined by optical rotation values in comparison to the value given in Ref. 3d,14.
12. Not published results of Julich Chiral Solutions GmbH.
13. Absolute configuration was determined by optical rotation: +13.4 (*c* 19, ethanol), compare also Talma, A. G.; Jouin, P.; De Vries, J. G.; Troostwijk, C. B.; Werumeus Buning, G. H.; Waninge, J. K.; Visscher, J.; Kellogg, R. M. *J. Am. Chem. Soc.* **1985**, *107*, 3981; and Kasai, M. *J. Org. Chem.* **1983**, *48*, 459.
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16. In 7 L of 100 mM Tris buffer (100 mM, pH 7.5), 167 mL 1,1,1-trifluoroacetone, 35 kU ADH LB (5 U/mL), 14 kU GDH BM (2 U/mL), 455 g glucose (300 mM) and 0.55 $NADP^+$ (0.1 mM) were dissolved and stirred for 70 h at 15 °C. The pH was adjusted by titration with 4 M NaOH. The reaction was stopped by ultrafiltration. After extraction the product was purified by distillation. The product (***R***)-**6** formed an azeotrope with *tert*-butyl methyl ether (75:25 by NMR) at 73 °C.
17. In 7 L of 100 mM Tris buffer (100 mM, pH 7.0), 167 mL 1,1,1-trifluoroacetone, 35 kU ADH RS1 (5 U/mL), 21 kU FDH CB (3 U/mL), 238 g sodium formate (500 mM) and 1 g NAD^+ (0.2 mM) were dissolved and stirred for 140 h at 15 °C. The pH was adjusted by titration with formic acid. The reaction was stopped by ultrafiltration. After extraction the product was purified by distillation. The product (***S***)-**6** formed an azeotrope with *tert*-butyl methyl ether (75:25) at 73 °C.