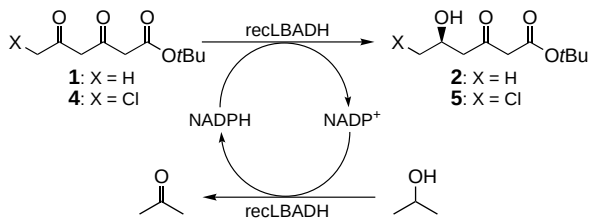
Highly Regio- and Enantioselective Reduction of 3,5-Dioxocarboxylates**

Michael Wolberg, Werner Hummel, Christian Wandrey, and Michael Müller*

3,5-Dioxocarboxylates (β,δ-diketo esters) unify the substructures of β-diketones and β-keto esters in an overlapping manner within a single molecule. While β-diketones and β-keto esters can be reduced with excellent enantioselectivity by metal-catalyzed hydrogenation[1] and microbial methods,[2] this does not likewise hold for 3,5-dioxocarboxylates. The question of regioselectivity must additionally be addressed if only one keto group of a 3,5-dioxocarboxylate is to be reduced. Chiral ruthenium catalysts were applied for this purpose and gave respectable, albeit improvable, results.[3] Clearly, 3,5-dioxocarboxylates represent a class of exceptionally demanding substrates with respect to regio- and enantioselective reduction and hence require particularly sophisticated catalysts. Alcohol dehydrogenases are such catalysts,[4] and indeed two publications related to biocatalytic reduction of 3,5-dioxocarboxylates report on enhanced enantioselectivities, albeit without simultaneous improvement of regioselectivity in the case of single-site reduction.[5] Nevertheless, we reasoned that the application of isolated alcohol dehydrogenases is highly promising when both high enantioselectivity *and* high regioselectivity are desired.

Herein we present a hitherto unprecedented highly enantioselective reduction of 3,5-dioxocarboxylates exclusively at position C-5 (δ). The resulting 5-hydroxy-3-oxocarboxylates are valuable intermediates in the synthesis of chiral building blocks such as 3,5-dihydroxycarboxylates[6] and β-keto δ-lactones. These compounds, in turn, have found frequent application in the stereoselective synthesis of pharmaceuticals and natural products.[7]

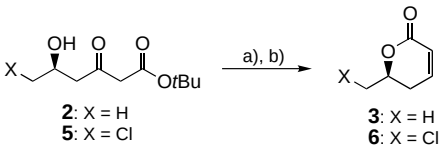
On photometrically screening the NAD(P)H consumption of several reductases in the presence of alkyl 3,5-dioxocarboxylates we found an NADP-dependent alcohol dehydrogenase of *Lactobacillus brevis* (LBADH) that accepts these compounds as substrates. From the viewpoint of synthetic organic chemistry, it is noteworthy that LBADH can be efficiently overexpressed in a recombinant *Escherichia coli* strain and used in the form of a crude cell extract (recLBADH).[8] Preparative-scale reduction of *tert*-butyl 3,5-dioxohexanoate (**1**)[9] with recLBADH gave the known



Scheme 1. Enzymatic reduction of 3,5-dioxocarboxylates and substrate-coupled recycling of the cofactor. **2**: 99.4% *ee*, 77%; **5**: >99.5% *ee*, 72%.

hydroxy keto ester **2**[10] with 99.4% *ee* and complete regioselectivity (Scheme 1). Neither the regioisomeric 3-hydroxy-5-oxohexanoate was detected by GC-MS and NMR spectroscopy nor were 3,5-dihydroxyhexanoates. The product **2**, which we used in the synthesis of (*R*)-*semi*-vioxanthin,[11] is not a substrate for LBADH with regard to reduction of the second keto group, as was determined by photometry.

For determination of the enantiomeric excess, hydroxy keto ester **2** was transformed into (*R*)-parasorbic acid **3**, the two enantiomers of which are easily separable by analytical HPLC on a chiral stationary phase (Scheme 2).[12]



Scheme 2. Synthesis of α,β-unsaturated δ-lactones **3** and **6**. a) $NaBH_4$, EtOH, 0 °C (quant.); b) cat. TsOH, toluene, Δ (60–70%).

To further increase the usefulness of this new enzymatic reduction as a synthetic method, a functional group was introduced at C-6 of the substrate (Scheme 1). We chose a chloro substituent because it is a generally exploitable functional group. Compared to its unchlorinated analogue **1**, the chloro derivative **4**[13] is accepted by LBADH with equally good activity. The product of the enzymatic reduction was identified as the expected *tert*-butyl (*S*)-6-chloro-5-hydroxy-3-oxohexanoate (**5**), and regio- and enantioselectivity were again outstanding (>99.5% *ee*, 72% yield of isolated product). The absolute configuration of the hydroxy keto ester **5** was established by independent synthesis of an authentic sample and comparison of the signs of the specific rotations.[14] The enantiomeric excess was determined after conversion to the chlorinated δ-lactone **6** as described above (Scheme 2).[12]

Hydroxy keto ester **5** is an advanced building block in the synthesis of a mevinic acid type HMG-CoA reductase inhibitor.[7g] Prasad's *syn*-selective reduction method[6a] was applied in that work. In our hands, this method gave quantitatively *syn*-configured (3*R*,5*S*)-3,5-dihydroxyhexanoate (**7**) with 93% *de* (Scheme 3). Furthermore, we found that Evans' *anti*-selective method[6b,c] resulted in the diastereomer (3*S*,5*S*)-3,5-dihydroxyhexanoate (**8**) with 90% *de* and quantitative yield. The diastereomeric excess of both diols can be readily increased to greater than 99% by a single recrystallization step (70% yield).[15]

[*] Dr. M. Müller, Dipl.-Chem. M. Wolberg, Prof. Dr. C. Wandrey
Institut für Biotechnologie 2
Forschungszentrum Jülich GmbH
52425 Jülich (Germany)
Fax (+49) 2461-61-3870
E-mail: mi.mueller@fz-juelich.de
Dr. W. Hummel
Institut für Enzymtechnologie
Heinrich-Heine-Universität Düsseldorf
40225 Düsseldorf (Germany)

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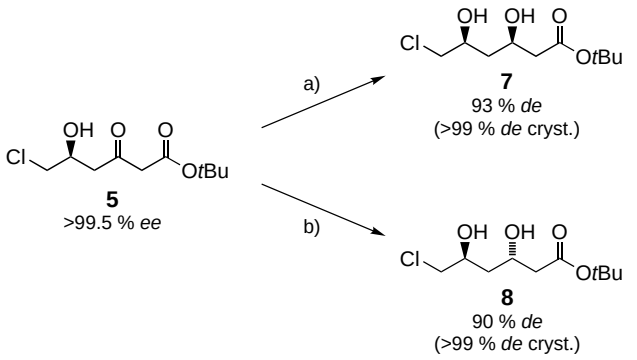

Scheme 3. Diastereoselective reduction of hydroxy keto ester **5**. a) 1. $B(OMe)Et_2$, THF/MeOH (80/20 v/v), −70 °C, 20 min; 2. $NaBH_4$, −70 °C, 3 h (quant.); b) $Me_4N[B(OAc)_3H]$, MeCN/AcOH (50/50 v/v), −25 °C, 5 h (quant.).

The hydroxy keto esters **2** and **5** were prepared in 77 and 72 % yield by using 0.3 – 1.0 mol % of $NADP^+$ (not optimized) and simple batch techniques. Advantageously, LBADH itself can recycle its cofactor by oxidation of 2-propanol, which is used as an additive (Scheme 1). The excess of 2-propanol is assumed to be the driving force of the reaction, and conversions of greater than 90 % were attained in each case, as was determined by GC-MS and NMR spectrosopy. The enzymatic reduction of the chlorinated diketo ester **4** can be easily scaled up and is routinely performed on a 75-g scale (8 L fed batch) in our laboratory.

In conclusion we have established the first highly regio- and enantioselective method to efficiently reduce polyketide-like compounds by applying isolated enzymes. At present, we are screening for biocatalysts with the opposite sense of enantioselectivity. Investigations concerning the scope of this new method, for example its applicability to dynamic kinetic resolution of C-4-substituted 3,5-dioxocarboxylates, are in progress.

Experimental Section

A solution of recLBADH was prepared by mechanically disrupting wet cells of recombinant *E. coli* strain recADH-HB101 + .[8] One unit (U) of enzyme activity is defined as the amount of recLBADH that catalyzes the oxidation of 1 μmol NADPH per minute when incubated with acetophenone (10 mM) and NADPH (0.25 mM) at 25 °C and pH 6.5 (100 mM phosphate buffer, 1 mM $MgCl_2$).

2: In a round-bottom flask, a solution of **1** (1.98 g, 9.9 mmol) in 2-propanol (5.1 mL, 66 mmol) was added to triethanolamine/HCl buffer (330 mL, 250 mM, pH 7.0) containing $MgCl_2$ (1 mM), and the mixture was vigorously stirred for 10 min. The stirring speed was adjusted to 60 rpm, and $NADP^+$ (28 mg, 33 μmol; FLUKA Nr. 93210, 90 %) and recLBADH (660 U) were added. After stirring for 24 h at room temperature, the solution was saturated with NaCl and extracted with ethyl acetate three times. The combined organic phases were dried over $MgSO_4$, the solvent was evaporated, and the crude product was purified by flash chromatography (silica, ethyl acetate/isohexane 40/60, v/v), to give hydroxy keto ester **2** (1.54 g; 77 %). The ^1H NMR spectrum was in agreement with published data.[10] ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C, only signals of the keto form are given): $\delta = 22.6$ (C-6), 28.1 ($C(CH_3)_3$), 51.2, 51.3 (C-2, C-4), 63.9 (C-5), 82.4 ($C(CH_3)_3$), 166.4 (C-1), 204.4 (C-3); $[\alpha]_D^{25} = -40.1$ ($c = 1.9$, $CHCl_3$), 99.4 % *ee*;[10] $[\alpha]_D^{26} = -39.6$ ($c = 2$, $CHCl_3$), 99 % *ee*.

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[12] DAICEL Chiracel OB (plus guard column), isohexane/2-propanol (80/20 v/v), 1.0 mL min^{-1}, 25 °C, 215 nm (UV-DAD). Retention times: (*S*)-**3**: 17.6, (*R*)-**3**: 23.8, (*R*)-**6**: 20.9, (*S*)-**6**: 24.3 min. In the case of the (*S*)-lactone derived from the enzymatic product **5**, none of the *R* enantiomer could be detected.

[13] Prepared by acylation of the lithium bis-enolate of *tert*-butyl acetoacetate with methyl chloroacetate (THF, −60 °C, 70 % yield of isolated product after column chromatography).

[14] Prepared according to ref. [7g], starting from commercially available ethyl (*S*)-4-chloro-3-hydroxybutyrate (Aldrich, 97 % *ee*). $[\alpha]_D^{25} = -23.0$ ($c = 1.5$, $CHCl_3$), 97 % *ee*; enzymatic product **5**: $[\alpha]_D^{25} = -24.9$ ($c = 1.4$, $CHCl_3$), > 99.5 % *ee*; ^1H NMR (300 MHz, $CDCl_3$, 20 °C, only

signals of the keto form are given): $\delta = 4.31$ (m, 1H; C*H*OH), 3.62 (dd, $J = 11.2$, 5.1 Hz, 1H; H-6), 3.57 (dd, $J = 11.2$, 5.0 Hz, 1H; H-6), 3.41 (s, 2H; H-2), 3.10 (br s, 1H; OH), 2.90 (dd, $J = 17.5$, 5.0 Hz, 1H; H-4), 2.83 (dd, $J = 17.5$, 7.3 Hz, 1H; H-4), 1.47 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C, only signals of the keto form are given): $\delta =$ 28.1 ($C(CH_3)_3$), 46.6, 48.4, 51.3 (C-2, C-4, C-6), 67.6 (C-5), 82.7 ($C(CH_3)_3$), 166.2 (C-1), 202.9 (C-3).

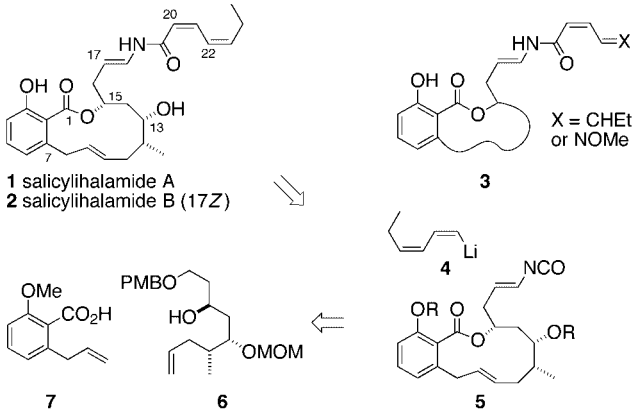
[15] The diastereomeric excess was determined by GC after formation of the corresponding acetonides (2,2-dimethoxypropane, cat. camphorsulfonic acid).

Revision of the Absolute Configuration of Salicylihalamide A through Asymmetric Total Synthesis**

Yusheng Wu, Lothar Esser, and Jef K. De Brabander*

Natural products that elicit a specific and unique biological response in mammalian cells represent valuable tools to identify, study, and target possible new gene products. In this context, the recent isolation of salicylihalamides A and B (**1** and **2**, Scheme 1) from the marine sponge *Haliclona* sp. is noteworthy.[1] Pattern-recognition analysis of their unique differential 60-cell mean-graph screening profiles (National Cancer Institute) suggests that the salicylihalamides belong to a potentially new mechanistic class of antitumor compounds.[1] Since their discovery in 1997, an emerging class of novel bioactive metabolites have been isolated that structurally relate to the salicylihalamides by virtue of an unprecedented highly unsaturated enamide attached to a macrocyclic salicylate (generalized structure **3**, Scheme 1). These include the mechanistically related lobatamides,[2] the potent cytostatic apicularens,[3] and selective inhibitors of oncogene-transformed cells (oximidines),[4] as well as compounds that induce low density lipoprotein (LDL) receptor gene expression.[5] The opportunity to develop chemistry in this area—none of these compounds have been synthesized previously—as well as to access variants for mode of action studies, prompted us to initiate a synthetic program towards this intriguing class of natural products.[6] Herein, we disclose the total synthesis of **1** and demonstrate unequivocally that the absolute configuration of natural (−)-salicylihalamide A, formulated as **1** through Mosher's ester ^1H NMR spectroscopic experiments by the group who isolated it,[1] was misassigned.

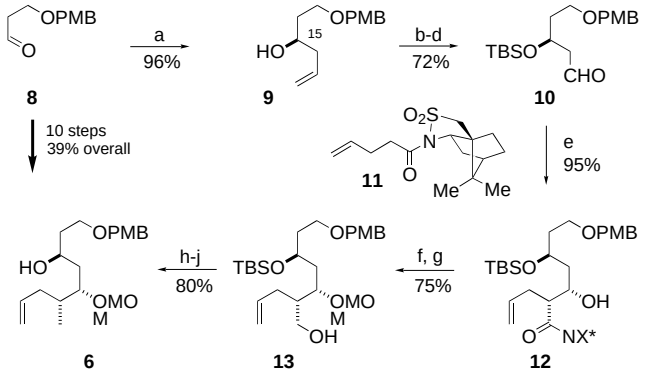
From the onset, we deemed it crucial to introduce the sensitive *N*-(alkenyl)heptadienamide side chain at a late stage in the synthesis (Scheme 1). Considering the options, we felt



Scheme 1. Synthetic strategy for salicylihalamide A. PMB = *para*-methoxybenzyl, MOM = methoxymethyl.

that the addition of 1-lithio-1,3-hexadiene (**4**) to isocyanate **5** would offer the distinct advantage of mild reaction conditions and control of stereochemistry.[7] Isocyanate **5** was to be derived from the corresponding *E*-α,β-unsaturated carboxylic acid (acyl azide formation/Curtius rearrangement), in turn accessible from a C17 aldehyde by Horner–Wadsworth–Emmons homologation. A Mitsunobu esterification/olefin ring-closing metathesis (RCM) tactic would ultimately unravel the 12-membered benzolactone ring into its primary components, polyol fragment **6** and benzoic acid derivative **7**.

A fully optimized procedure, delivering gram quantities of alcohol **6**, is presented in Scheme 2. We opted for an



Scheme 2. Reagents and conditions: a) 2-^{d}Icr$_2$B(allyl),[8] Et_2O, −78 °C, then NaOOH, 96%; b) TBSCl, imidazole, DMAP, DMF, 94%; c) cat. OsO_4, NMO, acetone/H_2O; d) $Pb(OAc)_4$, pyridine, PhH, 77% (steps c, d); e) **11**, $TiCl_4$, *i*Pr_2NEt, CH_2Cl_2, −78 °C, then **10**, −78 °C, 95%; f) MOMCl, NaI, *i*Pr_2NEt, CH_2Cl_2, 91%; g) $LiEt_3BH$, THF, −78 °C → RT, 82%; h) TsCl, Et_3N, DMAP, CH_2Cl_2, 91% (5% recovery of **13**); i) $LiEt_3BH$, THF, −78 °C → RT, 90%; j) TBAF, THF, 98%. Icr = isocaranyl, TBS = *tert*-butyldimethylsilyl, DMAP = 4-dimethylaminopyridine, DMF = dimethylformamide, NMO = 4-methylmorpholine *N*-oxide, RT = room temperature, Ts = tosyl = toluene-4-sulfonyl, TBAF = tetrabutylammonium fluoride, X* = boranesultam.

[*] Prof. Dr. J. K. De Brabander, Dr. Y. Wu, Dr. L. Esser[+]
Department of Biochemistry
University of Texas Southwestern Medical Center at Dallas
5323 Harry Hines Boulevard, Dallas, TX 75390-9038 (USA)
Fax: (+1) 214-648-6455
E-mail: jdebra@biochem.swmed.edu

[+] Author to whom correspondence regarding X-ray crystallographic analysis should be addressed. E-mail: esser@chop.swmed.edu

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