Biocatalytic Reduction of β,δ-Diketo Esters: A Highly Stereoselective Approach to All Four Stereoisomers of a Chlorinated β,δ-Dihydroxy Hexanoate

Michael Wolberg,[a] Werner Hummel,[b] and Michael Müller*[a]

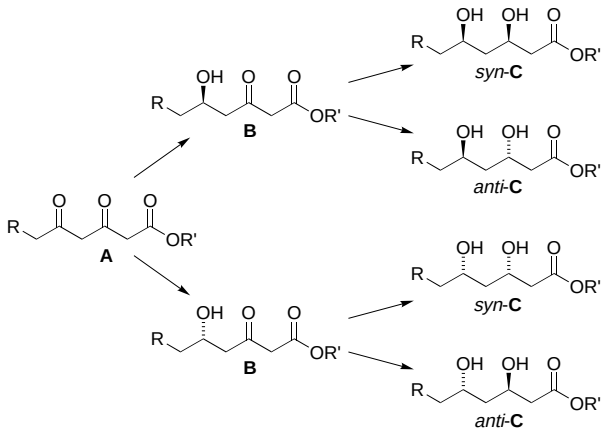
Abstract: A stereoselective chemoenzymatic synthesis of all four stereoisomers of *tert*-butyl 6-chloro-3,5-dihydroxyhexanoate (**6a**) is presented. The key step of the sequence is a highly regio- and enantioselective single-site reduction of *tert*-butyl 6-chloro-3,5-dioxohexanoate (**1a**) by two enantiocomplementary biocatalysts. Alcohol dehydrogenase from *Lactobacillus brevis* (recLBADH) afforded a 72% yield of enantiopure *tert*-butyl (*S*)-6-chloro-5-hydroxy-3-oxohexanoate [(*S*)-**2a**]. The enantiomer (*R*)-**2a** was prepared with 90–94% *ee* by Baker's yeast reduction in a biphasic system (50% yield). Both biotransformations were performed on a gram scale. The β-keto group of the enantiomeric δ-hydroxy-β-keto esters **2a** thus obtained was reduced by *syn*- and *anti*-selective borohydride reductions. Permutation of the reduction methods yielded all four stereoisomers of the crystalline target compound **6a** (≥99.3% *ee*, *dr* ≥ 205:1), which is a versatile 1,3-diol building block. recLBADH accepts a variety of β,δ-diketo esters as was determined in a photometric assay. *tert*-Butyl 3,5-dioxohexanoate (**1b**) and *tert*-butyl 3,5-dioxoheptanoate (**1c**) were reduced on a preparative scale as well to afford the corresponding δ-hydroxy-β-keto esters (*R*)-**2b** and (*R*)-**2c** with 99.4% *ee* and 98.1% *ee*, respectively.

Keywords: alcohols · asymmetric catalysis · enzymes · ketones · reduction

Introduction

Optically active β,δ-dihydroxy esters **C** have found widespread application in the stereoselective synthesis of natural products, polyol fragments, and chiral drugs.[1] Both the *syn*- and the *anti*-configured β,δ-dihydroxy esters **C** can be independently prepared from the corresponding chiral δ-hydroxy-β-keto esters **B** by means of highly diastereoselective reduction protocols.[2] Several syntheses of optically active hydroxy keto esters **B** have been described in the literature.[3] The regio- and enantioselective reduction of β,δ-diketo esters **A** is a straightforward and flexible approach. All four stereoisomers of a dihydroxy ester **C** can be derived from the same precursor **A** by this strategy (Scheme 1).

However, only a few publications dealing with this subject can be found in the literature. Hydrogenation of diketo esters **A** with chirally modified ruthenium catalysts resulted in mixtures of *syn*- and *anti*-dihydroxy esters **C** with varying enantiomeric excess.[4] A notable exception to this is represented by the recent work of Carpentier et al., who succeeded in controlling the reduction of methyl 3,5-dioxohexanoate (**1f**) at the initial step, namely the reduction of the β-keto group. The achieved enantiomeric excess was, nevertheless, limited to 78% at best.[4a] Highly enantioselective reduction of ethyl 6-benzyloxy-3,5-dioxohexanoate by ADH (alcohol dehydrogenase) of *Acinetobacter calcoaceticus* has been reported (97–>99% *ee*).[5] Regioselectivity was not encoun-



Scheme 1. Regio- and enantioselective reduction of β,δ-diketo esters **A**.

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tered, however, as was the case in the reduction of a variety of β,δ-diketo esters **A** with Baker's yeast.[6] The low to moderate enantiomeric excess (6–67% *ee*) of the prevailing β-hydroxy-δ-keto products suggests a mutual activity of at least two Baker's yeast enzymes with opposite enantioselectivities.

Nevertheless, the application of biocatalysis in a regio- and enantioselective reduction of β,δ-diketo esters **A** seemed most promising to us. Lack of selectivity in ADH catalyzed ketone reductions is the exception rather than the rule, especially when purified enzymes are applied.[7] In the present paper, we describe a highly regio- *and* enantioselective biocatalytic reduction of several β,δ-diketo esters **A**.[8] According to the strategy outlined in Scheme 1, all four stereoisomers of a chlorinated dihydroxy hexanoate **C** were synthesized in virtually enantiopure form.

Results and Discussion

Biocatalyst screening and enzymatic reduction of β,δ-diketo esters: In a photometric assay, that is, by monitoring NAD(P)H consumption at 340 nm, diketo esters **1a** and **1b** were added to ADH of *Lactobacillus brevis*, *Thermoanaerobium brockii*, horse liver, Baker's yeast, *Rhodococcus erythropolis*, and carbonyl reductase of *Candida parapsilosis*, all of which are known to be capable of reducing a variety of ketones.[7b, c] However, only NADP-dependent ADH of *Lactobacillus brevis* (LBADH) accepted diketo esters **1a** and **1b** as substrates. This stable enzyme is easily available in the form of a crude cell extract (recLBADH) from a recombinant *E. coli* strain.[9] As can be seen from Table 1, recLBADH accepts a broad range of β,δ-diketo esters **A**.

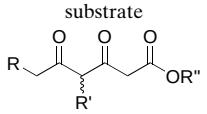
While the activity of recLBADH rapidly decreases on chain elongation at C-6 (compounds **1b–e**), the enzyme is less sensitive to variation of the ester group. All unsubstituted alkyl 3,5-dioxohexanoates are accepted with good activity (compounds **1b**, **f–l**), and only a slight preference for lipophilic ester alcohols can be noticed. The lower activity for hexyl ester **1j** is a consequence of the poor solubility of this substrate rather than a consequence of lower acceptance by the enzyme. Remarkably, the branched diketo ester *rac*-**1q** is also a good substrate for recLBADH. The *S* enantiomer of this compound is accepted preferentially, which allows a dynamic kinetic resolution.[10] Oxy-substitution at C-6 is not tolerated (compounds **1n–p**), in contrast to substitution by chlorine and fluorine (**1a** and **1m**).

To identify the keto group(s) reduced by recLBADH, we performed the reaction with diketo esters **1a–c** on a 1–10 millimole scale, by using substrate-coupled regeneration of NADPH. The cosubstrate 2-propanol was applied in excess to shift the equilibrium in the desired direction (Scheme 2).

Comparison of analytical data (^{1}H NMR spectroscopy, [α]) of the products (*R*)-**2b** and (*R*)-**2c** with literature data clearly proved the formation of *R*-configured δ-hydroxy-β-keto isomers of high optical purity (Table 2). In the case of the chlorinated product (*S*)-**2a**, authentic material was prepared by an independent route. Additionally, the enzymatic product (*S*)-**2a** was transformed into the known[11] β-keto δ-lactone (*S*)-**3** (Scheme 3). Neither the β-hydroxy-δ-keto regioisomer nor β,δ-dihydroxy esters could be detected by NMR and GC-MS analysis of the crude enzymatic products.

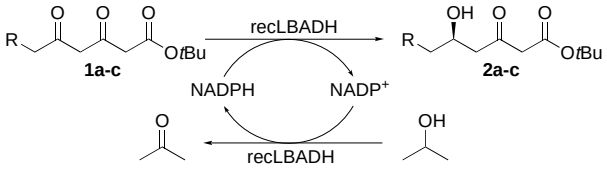
Precise determination of the enantiomeric excess was accomplished after transformation of hydroxy keto esters **2a–c** to α,β-unsaturated δ-lactones **4a–c** (Scheme 3), the

Table 1. Relative reaction rates of the reduction of β,δ-diketo esters by recLBADH (photometric assay).



	R	R′	R″	*c* [mmol L^{-1}]	rel. rate [%]
1a	Cl	H	*t*Bu	2[a]	77
1b	H	H	*t*Bu	10	86
1c	Me	H	*t*Bu	10[b]	18
1d	Et	H	*t*Bu	10[b]	4
1e	(*E*)-PhCH=CH	H	*t*Bu	0.5[b]	0
1f	H	H	Me	10	64
1g	H	H	Et	10	74
1h	H	H	*n*Pr	10	99
1i	H	H	allyl	10	93
1j	H	H	*n*hex	0.5[b]	54
1k	H	H	Bn	2	106
1l	H	H	*i*Pr	10	69
1m	F	H	*t*Bu	10	92
1n	MeO	H	Me	10	0
1o	MeO	H	*t*Bu	10	0
1p	BnO	H	Me	5	0
rac-**1q**	H	Me	*t*Bu	10	70
	acetophenone (standard)			10	100

[a] Higher concentration of substrate interferes at 340 nm. [b] Substrate not completely dissolved.

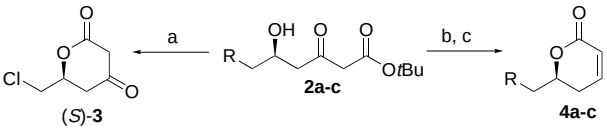


Scheme 2. Reduction of β,δ-diketo esters **1a–c** by recLBADH (R = Cl, H, Me).

Table 2. Products of the recLBADH catalyzed reduction of diketo esters **1a–c**.

	R	yield [%]	*ee*[a] [%]	$[\alpha]_D^{25}$	ref. $[\alpha]_D$ [% *ee*]
(*S*)-**2a**	Cl	72	> 99.5	− 24.9	− 23.0 (> 97), ref. [12]
(*R*)-**2b**	H	77	99.4	− 40.1	− 39.6 (99), ref. [13a]
(*R*)-**2c**	Me	61	98.1	− 36.0	− 35.6 (99), ref. [13b]

[a] Determined by HPLC (Chiracel OB) at the stage of the δ-lactones **4a–c**.



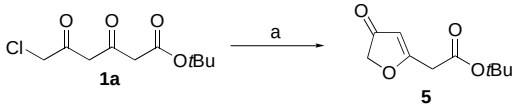
Scheme 3. Synthesis of δ-lactones **3** and **4a–c** (R = Cl, H, Me). a) cat. TsOH, CH_2Cl_2, RT, 4 d, 67%; b) $NaBH_4$, EtOH, 0 °C, 30 min; c) cat. TsOH, toluene, Δ, 2 h, 60–70%.

enantiomers of which are easily separable by HPLC (Chiralcel OB). In the case of the chlorinated lactone (*S*)-**4a**, the amount of *R* enantiomer was below the detection limit.

To the best of our knowledge, recLBADH is the first catalyst that allows the preparation of δ-hydroxy-β-keto esters by highly regio- *and* enantioselective reduction of β,δ-diketo esters. Additionally, the presented enzymatic reaction is of enormous preparative value. The substrates are readily available, and the reaction only requires a simple batch technique, which is easy to scale up. Reduction of the chlorinated compound **1a** is routinely performed on a 75 g scale in our laboratory (8 L fed batch).[14]

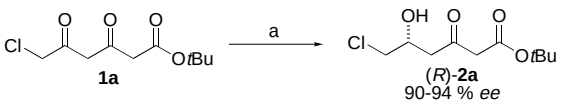
At the beginning of our investigations we were concerned about the susceptibility of diketo ester **1a** to furanone formation, which is a known reaction of other γ-chloro-β-diketones as well.[15] Indeed, stirring compound **1a** in mere pH 7.0 buffer/ethanol solution resulted in complete conversion to furanone **5** (Scheme 4).

We found this cyclization to be significantly suppressed in the enzymatic reduction of diketo ester **1a** by application of pH 5.5 buffer. The remaining fraction of by-product **5** reflects the high (>90%) yet incomplete enzymatic conversion. On the other hand, unreacted diketo ester **1a** would lower the enantiomeric excess in the subsequent diastereoselective borohydride reduction (see below). The formation of furanone **5** can be regarded as a convenient in situ elimination of unreacted starting material since it does not interfere with the borohydride reduction.



Scheme 4. Cyclization of diketo ester **1a**. a) phosphate buffer (pH 7.0)/ethanol (67:33 *v*/*v*), RT, 20 h, 79%.

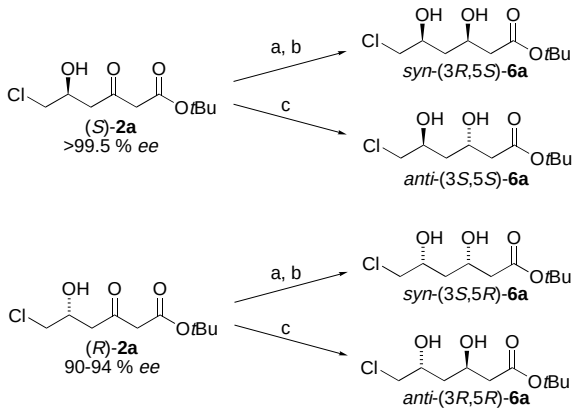
In order to synthesize the *R* enantiomer of hydroxy keto ester **2a**, we reinvestigated the Baker's yeast reduction of β,δ-diketo esters described by the Japanese working group at Mitsubishi Kasei.[6] In contrast to **1b** and **1c**, the chlorinated diketo ester **1a** was reduced with high regioselectivity at C-5 by Baker's yeast.[16] Only small amounts of the regioisomer and double-site reduction products (<5% total) could be detected by GC-MS. The enantiomeric excess (48%) of the product (*R*)-**2a** was greatly enhanced by application of a biphasic system (hexane/water or Amberlite XAD-7/water).[17] Furthermore, we tested cell preparations from different suppliers and found that a dried Baker's yeast gave the best results with regard to enantioselectivity and conversion.[18] A combination of the two parameters enhanced the enantiomeric excess of the product (*R*)-**2a** from 48% to 90–94% (Scheme 5).[19]



Scheme 5. Reduction of diketo ester **1a** by Baker's yeast. a) dried Baker's yeast, substrate **1a** adsorbed on XAD-7, water, RT, 24 h, 50%.

Application of the resin considerably facilitated the work-up, and formation of furanone **5** was found to be suppressed close to the detection limit, which is an additional benefit of the biphasic system. The reaction was carried out on a gram scale without difficulty.

Diastereoselective reduction of *tert*-butyl 6-chloro-5-hydroxy-3-oxo-hexanoate (2a): For the preparation of both enantiomers of dihydroxy ester *syn*-**6a** Prasad's *syn*-selective borohydride reduction was applied (Scheme 6).[2a, 20] This method



Scheme 6. Diastereoselective reduction of hydroxy keto esters (*S*)-**2a** and (*R*)-**2a**. a) 1. $B(OMe)Et_2$, THF/MeOH (80:20 *v*/*v*), −70°C, 20 min; 2. $NaBH_4$, −70°C, 3 h; b) 1. H_2O_2, THF/water (70:30 *v*/*v*, pH 9), 0°C → RT, 30 min; c) 1. $Me_4N[B(OAc)_3H]$, MeCN/AcOH (50:50 *v*/*v*), −25°C, 5 h.

quantitatively gave dihydroxy esters *syn*-**6a** in a diastereomeric ratio (*dr*) *syn*-**6a**/*anti*-**6a** = 28:1 to 45:1. The enantiomers of dihydroxy ester *anti*-**6a** were synthesized according to Evans' method,[2b] which resulted in a *dr* (*anti*-**6a**/*syn*-**6a**) = 14:1 to 18:1 (Scheme 6). Advantageously, all dihydroxy esters **6a** solidified, and a single crystallization step raised the diastereomeric ratios to ≥187:1. In the case of the two dihydroxy esters derived from the Baker's yeast reduction product most of the minor enantiomer was removed by this procedure as well. Further recrystallization lowered the amount of minor stereoisomers below the detection limit. Assignment of the relative configuration was supported by the known downfield shift of the carbinolic ^{13}C-resonances of *syn*-1,3-diols compared with those of the *anti*-diastereoisomers (Table 3).[2a, 21]

The diastereomeric ratios were determined by GC on chiral stationary phase (Cyclodex beta-1/P) at the stage of the acetonides **7a** (Scheme 7). All four stereoisomers can be separated by this method, and thus we could also investigate the enantiomeric excess of each individual dihydroxy ester **6a**. The *ee* thus determined for the crude products confirmed the values for (*S*)-**2a** and (*R*)-**2a**, which were measured at the stage of the lactones (*S*)-**4a** and (*R*)-**4a** by HPLC (see above).

Nucleophilic substitution of chlorine: A two-step conversion of acetonide (3*R*,5*S*)-**7a** to hydroxy compound (3*R*,5*S*)-**8** is known from the patent literature.[12a] This compound is an

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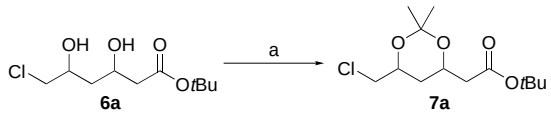
Table 3. Diastereoselective reduction of hydroxy keto esters (*S*)-**2a** (>99.5% *ee*) and (*R*)-**2a** (90–94% *ee*).

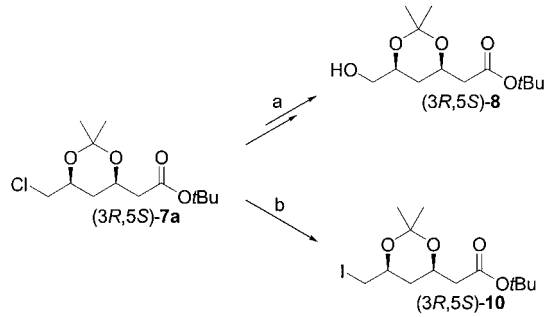
	scale [mmol]	*dr*[a] *syn*/*anti*	*ee*[a] [%]	yield [%]	δ ^{13}C C-3/C-5
syn-(3*R*,5*S*)-**6a**	86–217	205:1 (28:1)	>99.5	62	68.4/71.6
syn-(3*S*,5*R*)-**6a**	3–12	187:1 (45:1)[b]	98.0 (90.3)[b]	52	68.4/71.6
anti-(3*S*,5*S*)-**6a**	3	1:211 (1:14)	>99.5	70	65.5/68.9
anti-(3*R*,5*R*)-**6a**	1–4	1:316 (1:18)	99.3 (93.8)	68	65.5/68.9

[a] Diastereomeric ratio and enantiomeric excess of crystallized product and crude product (in brackets); determined by GC (Cyclodex beta-1/P) at the stage of the acetonide **7a**; "*syn*" and "*anti*" refer to the major pair of diastereomers homochiral at C-5. [b] *ee* > 99.5% and *dr* > 400:1 after recrystallization.



Scheme 7. Synthesis of acetonide **7a**. a) 2,2-Dimethoxy-propane, cat. CSA (camphorsulfonic acid), 25°C, 4–7 h, 100% conv.

advanced intermediate in the synthesis of HMG-CoA reductase inhibitors.[1j] Iodide (3*R*,5*S*)-**10** has been utilized for this purpose, too.[1i] We were able to substitute the chlorine of acetonide (3*R*,5*S*)-**7a** by iodine in a single step under advanced halogen exchange conditions (52% yield; Scheme 8).[22] However, conversion was incomplete, and



Scheme 8. Nucleophilic substitution of the chlorine of acetonide (3*R*,5*S*)-**7a**. a) see ref. [12a, b]; b) KI, [18]crown-6, toluene, Δ, 4 d, 52%.

the remaining starting material could not be removed from the product (3*R*,5*S*)-**10**. Furthermore, complete decomposition was encountered in several experiments.

Alternatively, epoxide (3*R*,5*S*)-**9** was regioselectively opened with lithium iodide on silica.[23] The crude product was immediately subjected to acetonide protection which afforded the desired iodide (3*R*,5*S*)-**10** with a 58% yield (44% from (3*R*,5*S*)-**6a**; Scheme 9).

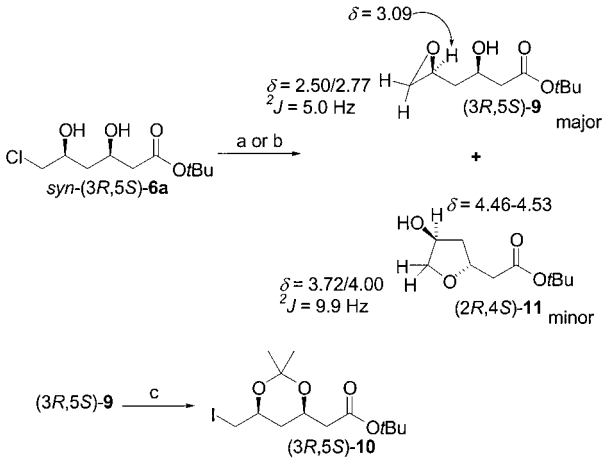
Epoxide (3*R*,5*S*)-**9** was easily obtained from dihydroxy ester *syn*-(3*R*,5*S*)-**6a** by treatment with DBU (1,8-diazobicyclo[5.4.0]undec-7-ene, 66% yield) or KOH (46% yield). The amount of tetrahydrofurane (2*R*,4*S*)-**11** from competing 5-*exo*-tet ring closure was limited to 10–13% in both experiments (^{1}H NMR analysis). The two resulting isomers were distinguished by correlation of their NMR data with data of closely related compounds.[24] The diagnostic proton resonances of the epoxides show a distinct upfield shift and difference in vicinal coupling compared with those of the isomeric tetrahydrofuranes (Figure 1).

Epoxy esters like (3*R*,5*S*)-**9** are also favorable intermediates in the synthesis of HMG-CoA reductase inhibitors since the epoxide can be regioselectively opened by carbon nucleophiles, for example, cuprates.[25]

Conclusion

All four stereoisomers of the chlorinated dihydroxy ester **6a** can be synthesized in an enantiopure form on a preparative scale by a flexible stereoselective two-step reduction sequence. For the crucial first step, two enantiocomplementary biocatalysts are employed, namely recLBADH and Baker's yeast, which allow the highly regio- and enantioselective reduction of the δ-keto group of diketo ester **1a**. The keto group of the products (*S*)-**2a** and (*R*)-**2a** can be reduced either *syn*-selective or *anti*-selective by application of known borohydride reduction protocols. The carbon chain of the resulting dihydroxy ester **6a** is terminated with two compatible functional groups of distinguishable reactivity, which makes this compound a valuable 1,3-diol building block.

The reduction of β,δ-diketo esters with ADH of *L. brevis* is a reaction of broad applicability. A great variety of diketo



Scheme 9. Synthesis and regioselective opening of epoxide (3*R*,5*S*)-**9**. a) DBU, CH_2Cl_2, Δ, 24 h, 66%; b) KOH, Et_2O, 0°C, 1 h, 46%; c) 1. LiI, SiO_2, RT, 1 h; 2. 2,2-dimethoxy-propane, cat. CSA, RT, 24 h, 58%.

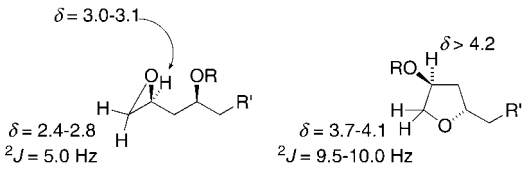


Figure 1. ^{1}H NMR spectroscopy of oxy-substituted epoxides and tetrahydrofuranes (relative configuration shown).[24]

esters are accepted by this readily available enzyme, and the required reaction technique is remarkably simple. As a result of the mild conditions, the reaction can be carried out without special care even in the case of sensitive compounds like **1a**. No serious limitations with regard to a further scale-up are apparent. The hydroxy keto esters thus available are compounds of high functionalization, and a variety of chemical modifications are known from the literature. We are currently investigating new applications of these compounds in the synthesis of chiral drugs and natural products.

Experimental Section

General: All solvents were HPLC grade or analytical grade. When necessary, solvents were dried prior to use: THF was distilled from sodium (benzophenone ketyl as indicator). Methanol and dichloromethane were stored over 4 Å molecular sieves. Acetonitrile was distilled from calcium hydride. Acetic acid was refluxed with acetic acid anhydride (3%) and then subjected to fractional distillation. Air- and/or moisture-sensitive reactions were carried out under an atmosphere of dry nitrogen in oven-dried glassware. Silica gel 60 F_{254} precoated aluminium sheets (Merck) were used for analytical thin layer chromatography. Visualization was accomplished by UV light or by dipping the plate into a solution of *p*-anisaldehyde (1 mL) in acetic acid/concd sulfuric acid (100 mL, 98:2 *v*/*v*) followed by heating. Flash chromatography was performed on silica gel 60 (40–63 μm, Merck). Acid-washed silica gel was used for *β*,*δ*-diketo esters.[26] Melting points were determined with a Büchi B-540 melting point apparatus. NMR spectra were recorded with a Bruker AMX-300 spectrometer at 20 °C. ^{1}H NMR: 300 MHz, residual undeuterated solvent as internal standard ($CHCl_3$: $\delta = 7.27$). ^{13}C NMR: 75.5 MHz, deuterated solvent as internal standard ($CDCl_3$: $\delta = 77.2$), broadband proton decoupling. Assignments were supported by additional DEPT-135 experiments. GC-MS analysis was carried out with a Hewlett-Packard HP 6890 GC system (HP-5MS column) coupled to an HP 5973 mass selective detector.[27] GC on chiral stationary phase was performed by a Chrompack CP 9002 equipped with a flame ionization detector. HPLC on chiral stationary phase was performed with a Hewlett-Packard HP 1100 system (UV-DAD). Optical rotations were determined with a Perkin-Elmer 241 polarimeter (1 dm cell). Mass spectroscopy (Kratos AEI MS50) and elemental analyses (Heraeus, Vario EL) were performed in the analytical department of the Kekulé Institut für Organische Chemie und Biochemie (Universität Bonn). Mass peaks with relative intensity $< 10\%$ are omitted unless they correspond to $[M]^+$ or some other informative fragment. Spectrophotometric enzyme assays were performed with a Pharmacia Ultrospec 2000 spectrophotometer in disposable cuvettes (1.5 mL, Brandt). Commercially available reagents were used as delivered unless otherwise stated. Tetramethylammonium triacetoxyborohydride and $NADP^+$ were purchased from Fluka, 2-chloro-*N*-methoxy-*N*-methylacetamide and Amberlite XAD-7 from Aldrich. Dried emulsifier-free Baker's yeast (Bio-Zentrale GmbH, Stubenberg, Germany) was purchased from a local supermarket.

Preparation of *β*,*δ*-diketo esters: Diketo esters **1f** and **1g** were prepared from dehydroacetic acid as described in the literature and purified by distillation.[28] Esters **1b**, **1i**, **1k**, and **1l** were prepared from acetoacetylated Meldrum's acid[29] as described in the literature and purified by Kugelrohr distillation and subsequent flash chromatography on acid-washed silica gel.[30] The derivatives **1h** and **1j** were obtained by the same method as follows.

***n*-Propyl 3,5-dioxohexanoate (1h)**: Acetocetylated Meldrum's acid (1.14 g, 5.0 mmol) and 1-propanol (0.90 g, 15 mmol) were refluxed in toluene (10 mL) for three hours. After removal of volatiles under reduced pressure, the residue was treated with dichloromethane and filtered. The filtrate was concentrated under reduced pressure, and the residue was subjected to Kugelrohr distillation (0.1 mbar, 70 °C). Flash chromatography of the distillate on acid-washed silica gel (ethyl acetate/*n*-hexane 20:80 *v*/*v*) gave title compound **1h** as a light-yellow oil. Yield: 0.45 g (48%).

^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): ratio enol form (ef): keto form (kf) = 89:11; $\delta = 0.93$ (t, $J = 7.4$ Hz, 3 H_{ef+kf}; CH_2CH_3), 1.66 (m, 2 H_{ef+kf}; CH_2CH_3), 2.07 (s, 3 H_{ef}; H-6), 2.25 (s, 3 H_{kf}, H-6), 3.33 (s, 2 H_{ef}; H-2), 3.55 (s, 2 H_{kf}; H-2), 3.74 (s, 2 H_{kf}; H-4), 4.08 (t, $J = 6.7$ Hz, 2 H_{kf}; OCH_2) overlapping with 4.09 (t, $J = 6.7$ Hz, 2 H_{ef}; OCH_2), 5.61 (s, 1 H_{ef}; H-4), 15.11 (brs, 1 H_{ef}; OH); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): enol form: $\delta = 10.4$ (CH_2CH_3), 22.0 (CH_2CH_3), 24.5 (C-6), 45.3 (C-2), 67.3 (OCH_2), 100.7 (C-4), 167.8 (C-1), 187.4, 190.3 (C-3, C-5); MS (70 eV, EI): *m*/*z* (%): 186 (21) $[M]^+$, 144 (27), 129 (19), 127 (29) $[M - OnPr]^+$, 126 (43), 111 (15), 102 (13), 98 (32), 85 (100) $[M - CH_2COOnPr]^+$, 84 (16), 69 (13); HRMS (EI) calcd for $C_9H_{14}O_4$ $[M]^+$: 186.0892; found: 186.0902.

***n*-Hexyl 3,5-dioxohexanoate (1j)**: Prepared from acetoacetylated Meldrum's acid (1.14 g, 5.0 mmol) and 1-hexanol (1.53 g, 15 mmol) as described in the example above. Kugelrohr distillation (10^{-3} mbar, 120 °C) and subsequent flash chromatography on acid-washed silica gel (ethyl acetate/*n*-hexane 20:80 *v*/*v*) gave title compound **1j** as a light-yellow oil. Yield: 0.59 g (52%).

^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): ratio enol form (ef):keto form (kf) = 83:17; $\delta = 0.87$ (brt, $J = 6.9$ Hz, 3 H_{ef+kf}; CH_2CH_3), 1.23–1.39 (m, 6 H_{ef+kf}; $(CH_2)_3CH_2CH_3$), 1.58–1.68 (m, 2 H_{ef+kf}; CH_2CH_3), 2.06 (s, 3 H_{ef}; H-6), 2.25 (s, 3 H_{kf}; H-6), 3.32 (s, 2 H_{ef}; H-2), 3.54 (s, 2 H_{kf}; H-2), 3.74 (s, 2 H_{kf}; H-4), 4.11 (t, $J = 6.8$ Hz, 2 H_{kf}; OCH_2) overlapping with 4.12 (t, $J = 6.7$ Hz, 2 H_{ef}; OCH_2), 5.60 (s, 1 H_{ef}; H-4), 15.10 (brs, 1 H_{ef}; OH); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): enol form: $\delta = 14.1$ (CH_2CH_3), 22.7 (CH_2CH_3), 24.5 (C-6), 25.6, 28.5, 31.5 ($(CH_2)_3CH_2CH_3$), 45.3 (C-2), 65.8 (OCH_2), 100.7 (C-4), 167.8 (C-1), 187.4, 190.3 (C-3, C-5); MS (70 eV, EI): *m*/*z* (%): 228 (23) $[M]^+$, 186 (10), 145 (35), 129 (24), 127 (54) $[M - OnHex]^+$, 126 (67), 111 (10), 103 (18), 102 (15), 98 (27), 85 (100) $[M - CH_2COOnHex]^+$, 84 (13), 56 (10), 55 (10); HRMS (EI) calcd for $C_{12}H_{20}O_4$ $[M]^+$: 228.1362; found: 228.1370.

Diketo esters a) **1a**, **1c**, **1d**, **1e**,[31] **1q**[10] and b) **1n**, **1o**, **1p**,[4b] were prepared by acylation of acetoacetate Na,Li-bisenolates[32] with a) Weinreb amides[33] and b) methyl 2-alkoxyacetates. Alternatively, chlorinated diketo ester **1a** was synthesized by acylation of *tert*-butyl acetoacetate Li,Li-bisenolate with methyl 2-chloroacetate. Fluoro compound **1m** was prepared in a similar manner with TMEDA (*N*,*N*,*N*′,*N*′-tetramethyl 1,2-ethanediamine) additionally present.[34]

General procedure for the acylation of acetoacetate Na,Li-bisenolates: Acetoacetate was added dropwise ($T_{max} = 10$ °C) to an ice-cooled suspension of sodium hydride (60% suspension in oil) in anhydrous THF, and the mixture was stirred for an additional ten minutes. The clear solution was cooled to −10 °C, and *n*-butyl lithium (1.6 mol L^{-1} in *n*-hexane) was added dropwise ($T_{max} = 0$ °C). After stirring for an additional 10 min, the acylation reaction was carried out as indicated. Hydrolysis was performed by pouring the solution into a vigorously stirred ice-cooled mixture (50:50) of ethyl acetate and hydrochloric acid (2 mol L^{-1}, HCl (4–6 equiv) with regard to the bisenolate). The aqueous phase was separated and extracted twice with ethyl acetate. The unified organic phases were washed with aq. $NaHCO_3$ (5%), water, and brine. After drying over Na_2SO_4, the solvent was removed under reduced pressure, and the product was purified as indicated.

***tert*-Butyl 6-chloro-3,5-dioxohexanoate (1a)**

Method A: A solution of Na,Li-bisenolate was prepared as described in the general procedure from *tert*-butyl acetoacetate (0.79 g, 5.0 mmol), sodium hydride (0.20 g of a 60% suspension in oil, 5.0 mmol), and *n*-butyl lithium (3.1 mL of a 1.6 mol L^{-1} solution in *n*-hexane, 5.0 mmol) in THF (15 mL). The solution was cooled to −75 °C, and a solution of 2-chloro-*N*-methoxy-*N*-methylacetamide (0.69 g, 5.0 mmol) in anhydrous THF (4 mL) was syringed into the flask ($T_{max} = -60$ °C). The solution was stirred at this temperature for 45 minutes, warmed to −30 °C over a period of 30 minutes, stirred for an additional 15 minutes at this temperature, and then recooled to −75 °C. Workup was carried out as described in the general procedure. Flash chromatography on acid-washed silica gel (ethyl acetate/*n*-hexane 20:80 *v*/*v*) gave title compound **1a** as an light-yellow oil. Yield: 0.68 g (58%).

^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): ratio enol form (ef):keto form (kf) = 88:12; $\delta = 1.48$ (s, 9 H_{ef+kf}; $C(CH_3)_3$), 3.31 (s, 2 H_{ef}; H-2), 3.49 (s, 2 H_{kf}; H-2), 3.92 (s, 2 H_{kf}; H-4), 4.06 (s, 2 H_{ef}; H-6), 4.21 (s, 2 H_{kf}; H-6), 5.97 (s, 1 H_{ef}; H-4), 14.76 (brs, 1 H_{ef}; OH); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): enol form: $\delta = 28.1$ ($C(CH_3)_3$), 44.4, 46.0 (C-2, C-6), 82.6 ($C(CH_3)_3$), 98.9 (C-4), 166.5 (C-1), 187.0, 187.3 (C-3, C-5); MS (70 eV, EI): *m*/*z* (%): 234 (1) $[M]^+$, 198 (6) $[M - HCl]^+$, 179 (10), 161/163 (30/10) $[M - OtBu]^+$, 129 (26), 119/121 (34/11) $[M - CH_2COOtBu]^+$, 103 (15), 85 (11), 57 (100) $[C_4H_9]^+$.

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Method B: *tert*-Butyl acetoacetate (7.9 g, 50 mmol) was added dropwise to a solution of LDA [prepared from diisopropyl amine (10.6 g, 105 mmol) and *n*-butyl lithium (64 mL of a 1.6 mol L^{-1} solution in *n*-hexane, 102 mmol) in 300 mL THF] in THF at −15 °C, and the solution was stirred at this temperature for ten minutes after addition was complete. The solution was cooled to −75 °C and methyl 2-chloroacetate (5.4 g, 50 mmol) was added dropwise ($T_{max} = -65$ °C). The solution was stirred at this temperature for 25 minutes after addition was complete. Workup was carried out as described in the general procedure, and unreacted starting materials were removed in vacuo. A yellow oil was obtained (11.0 g, 94 %) that consisted mainly (approx. 95 %) of the desired product **1a** as determined by ^{1}H NMR spectroscopy.

***tert*-Butyl 3,5-dioxoheptanoate (1c)**

a) *N-Methoxy-N-methylpropionamide*: Pyridine (1.69 mL, 21 mmol) at 0 °C was added dropwise to a solution of *O*,*N*-dimethyl-hydroxylamine hydrochloride (1.02 g, 10.5 mmol) and propionyl chloride (0.93 g, 10 mmol) in anhydrous dichloromethane (50 mL). The solution was stirred at room temperature for 3.5 h, washed with hydrochloric acid (0.5 mol L^{-1}) twice, and concentrated under reduced pressure to a volume of approximately 20 mL. Diethyl ether (30 mL) was added, and the solution was washed with aq. $NaHCO_3$ (5 %) and brine. After drying over $MgSO_4$, the solvent was removed under reduced pressure, and the amide was left as a colorless oil that was used for acylation without further purification. Yield: 1.04 g (89 %).

^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): $\delta = 1.09$ (t, $J = 7.5$ Hz, 3H; H-3), 2.40 (q, $J = 7.5$ Hz, 2H; H-2), 3.13 (s, 3H; NCH_3), 3.64 (s, 3H; OCH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): $\delta = 8.8$ (C-3), 25.3 (C-2), 32.3 (NCH_3), 61.2 (OCH_3), 174.9 (C-1); MS (70 eV, EI): *m*/*z* (%): 117 (17) $[M]^+$, 87 (10), 61 (100) $[NH(OMe)Me]^+$, 57 (97).

b) *Acylation reaction*: A solution of Na,Li-bisenolate was prepared as described in the general procedure from *tert*-butyl acetoacetate (0.79 g, 5.0 mmol), sodium hydride (0.20 g of a 60 % suspension in oil, 5.0 mmol), and *n*-butyl lithium (3.1 mL of a 1.6 mol L^{-1} solution in *n*-hexane, 5.0 mmol) in THF (20 mL). *N*-Methoxy-*N*-methylpropionamide (0.59 g, 5.0 mmol) was syringed into the flask at −10 °C. The solution was stirred at this temperature for 15 minutes, warmed to room temperature, and stirred for an additional 30 minutes. Workup was carried out as described in the general procedure. After removal of unreacted *tert*-butyl acetoacetate by Kugelrohr distillation (0.2 mbar, 40 °C), the residue was subjected to flash chromatography on acid-washed silica gel (ethyl acetate/*n*-hexane 15/85 *v*/*v*); this yielded title compound **1c** (0.78 g, 73 %) as a light-yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): ratio enol form (ef):keto form (kf) = 82:18; $\delta = 1.07$ (t, $J = 7.3$ Hz, $3H_{kf}$; H-7), 1.15 (t, $J = 7.5$ Hz, $3H_{ef}$; H-7), 1.47 (s, $9H_{ef+kf}$; $C(CH_3)_3$), 2.35 (q, $J = 7.5$ Hz, $2H_{ef}$; H-6), 2.55 (q, $J = 7.3$ Hz, $2H_{kf}$; H-6), 3.25 (s, $2H_{ef}$; H-2), 3.48 (s, $2H_{kf}$; H-2), 3.73 (s, $2H_{kf}$; H-4), 5.60 (s, $1H_{ef}$; H-4), 15.15 (br s, $1H_{ef}$; OH); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): enol form: $\delta = 9.7$ (C-7), 28.1 ($C(CH_3)_3$), 31.2 (C-6), 46.4 (C-2), 82.1 ($C(CH_3)_3$), 99.2 (C-4), 167.0 (C-1), 187.4, 194.7 (C-3, C-5); MS (70 eV, EI): *m*/*z* (%): 214 (2) $[M]^+$, 159 (12), 158 (29), 141 (36) $[M - OtBu]^+$, 140 (17), 129 (40), 111 (13), 99 (52) $[M - CH_2COOtBu]^+$, 85 (14), 84 (10), 57 (100) $[C_4H_9]^+$.

***tert*-Butyl 3,5-dioxooctanoate (1d)**

a) *N-Methoxy-N-methylbutyramide*: This compound was prepared as described in the preceding example from *O*,*N*-dimethyl-hydroxylamine hydrochloride (1.02 g, 10.5 mmol), butyryl chloride (1.07 g, 10 mmol), and pyridine (1.69 mL, 21 mmol) with 89 % yield.

^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): $\delta = 0.94$ (t, $J = 7.4$ Hz, 3H; H-4), 1.64 (sext, $J = 7.4$ Hz, 2H; H-3), 2.38 (t, $J = 7.4$ Hz, 2H; H-2), 3.16 (s, 3H; NCH_3), 3.66 (s, 3H; OCH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): $\delta = 14.0$ (C-4), 18.2 (C-3), 32.2 (NCH_3), 33.9 (C-2), 61.3 (OCH_3), 174.7 (C-1); MS (70 eV, EI): *m*/*z* (%): 131 (20) $[M]^+$, 71 (98), 61 (100) $[NH(OMe)Me]^+$.

b) *Acylation reaction*: A solution of Na,Li-bisenolate was prepared as described in the general procedure from *tert*-butyl acetoacetate (0.63 g, 4.0 mmol), sodium hydride (0.18 g of a 60 % suspension in oil, 4.5 mmol), and *n*-butyl lithium (2.6 mL of a 1.6 mol L^{-1} solution in *n*-hexane, 4.2 mmol) in THF (30 mL). *N*-Methoxy-*N*-methylbutyramide (0.54 g, 4.1 mmol) was syringed into the flask at −10 °C. The solution was stirred at this temperature for 15 minutes, warmed to room temperature, and stirred for an additional 60 minutes. Workup was carried out as described in the general procedure. Kugelrohr distillation (0.04 mbar, 80 °C) gave title compound **1d** as a light-yellow oil. Yield: 0.64 g (70 %).

^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): ratio enol form (ef):keto form (kf) = 86:14; $\delta = 0.91$ (t, $J = 7.4$ Hz, $3H_{kf}$; H-8) overlapping with 0.94 (t, $J = 7.4$ Hz, $3H_{ef}$; H-8), 1.46 (s, $9H_{ef+kf}$; $C(CH_3)_3$), 1.61 (m, $2H_{ef+kf}$; H-7), 2.26 (t, $J = 7.5$ Hz, $2H_{ef}$; H-6), 2.49 (t, $J = 7.4$ Hz, $2H_{kf}$; H-6), 3.24 (s, $2H_{ef}$; H-2), 3.46 (s, $2H_{kf}$; H-2), 3.70 (s, $2H_{kf}$; H-4), 5.58 (s, $1H_{ef}$; H-4), 15.18 (br s, $1H_{ef}$; OH); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): enol form: $\delta = 13.8$ (C-8), 19.3 (C-7), 28.1 ($C(CH_3)_3$), 39.8 (C-6), 46.7 (C-2), 82.0 ($C(CH_3)_3$), 99.9 (C-4), 167.0 (C-1), 188.2, 193.1 (C-3, C-5); MS (70 eV, EI): *m*/*z* (%): 228 (1) $[M]^+$, 173 (11), 172 (32), 155 (43) $[M - OtBu]^+$, 154 (19), 144 (41), 129 (64), 113 (78) $[M - CH_2COOtBu]^+$, 111 (21), 85 (10), 84 (17), 71 (42), 69 (11), 57 (100) $[C_4H_9]^+$.

Methyl 6-methoxy-3,5-dioxo-hexanoate (1n): A solution of Na,Li-bisenolate was prepared as described in the general procedure from methyl acetoacetate (0.70 g, 6.0 mmol), sodium hydride (0.24 g of a 60 % suspension in oil, 6.0 mmol), and *n*-butyl lithium (3.8 mL of a 1.6 mol L^{-1} solution in *n*-hexane, 6.1 mmol) in THF (30 mL). The solution was cooled to −75 °C, and methyl 2-methoxyacetate (0.31 g, 3.0 mmol) was added in one portion. The solution was warmed to 0 °C and stirred for two hours at this temperature. Workup was carried out as described in the general procedure. Kugelrohr distillation (10^{-1} mbar, 75 °C) and flash chromatography on acid-washed silica gel (ethyl acetate/*n*-hexane 30:70 *v*/*v*) gave title compound **1n** as a yellow oil. Yield: 0.14 g (25 %).

^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): ratio enol form (ef):keto form (kf) = 85:15; $\delta = 3.38$ (s, $2H_{ef}$; H-2), 3.39 (s, $3H_{kf}$; CH_2OCH_3), 3.41 (s, $3H_{ef}$; CH_2OCH_3), 3.58 (s, $2H_{kf}$; H-2), 3.73 (s, $3H_{ef+kf}$, $COOCH_3$), 3.76 (s, $2H_{kf}$; H-4), 4.00 (s, $2H_{ef}$; H-6), 4.02 (s, $2H_{kf}$; H-6), 5.88 (s, $1H_{ef}$; H-4), 14.95 (br s, $1H_{ef}$; OH); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): enol form: $\delta = 44.8$ (C-2), 52.6 ($COOCH_3$), 59.5 (CH_2OCH_3), 73.3 (C-6), 97.8 (C-4), 168.0 (C-1), 186.7, 191.0 (C-3, C-5); MS (70 eV, EI): *m*/*z* (%): 188 (2) $[M]^+$, 158 (10), 143 (100) $[M - CH_2OCH_3]^+$, 115 (24) $[M - CH_2COOMe]^+$, 111 (15), 101 (63), 69 (31), 69 (13).

***tert*-Butyl 6-methoxy-3,5-dioxohexanoate (1o)**: A solution of Na,Li-bisenolate was prepared as described in the general procedure from *tert*-butyl acetoacetate (1.58 g, 10.0 mmol), sodium hydride (0.40 g of a 60 % suspension in oil, 10.0 mmol), and *n*-butyl lithium (6.3 mL of a 1.6 mol L^{-1} solution in *n*-hexane, 10.1 mmol) in THF (30 mL). The solution was cooled to −75 °C, and methyl 2-methoxyacetate (0.52 g, 5.0 mmol) was added in one portion. The solution was warmed to 0 °C over a period of 45 minutes and stirred for an additional two hours at this temperature. Workup was carried out as described in the general procedure. Kugelrohr distillation (10^{-2} mbar, 85 °C) and flash chromatography on acid-washed silica gel (ethyl acetate/*n*-hexane 20:80 *v*/*v*) gave title compound **1o** as a yellow oil. Yield: 0.91 g (79 %).

^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): ratio enol form (ef):keto form (kf) = 85:15; $\delta = 1.47$ (s, $9H_{ef+kf}$; $C(CH_3)_3$), 3.28 (s, $2H_{ef}$; H-2), 3.41 (s, $3H_{kf}$; OCH_3), 3.43 (s, $3H_{ef}$; OCH_3), 3.48 (s, $2H_{kf}$; H-2), 3.76 (s, $2H_{kf}$; H-4), 4.01 (s, $2H_{ef}$; H-6), 4.04 (s, $2H_{kf}$; H-6), 5.89 (s, $1H_{ef}$; H-4), 15.03 (br s, $1H_{ef}$; OH); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): enol form: $\delta = 28.2$ ($C(CH_3)_3$), 46.3 (C-2), 59.6 (OCH_3), 73.5 (C-6), 82.3 ($C(CH_3)_3$), 97.7 (C-4), 166.8 (C-1), 187.3, 191.2 (C-3, C-5); MS (70 eV, EI): *m*/*z* (%): 230 (1) $[M]^+$, 185 (18), 157 (21) $[M - OtBu]^+$, 144 (16), 129 (100), 115 (45) $[M - CH_2COOtBu]^+$, 111 (27), 84 (10), 57 (65) $[C_4H_9]^+$.

***tert*-Butyl 6-fluoro-3,5-dioxohexanoate (1m)**: *tert*-Butyl acetoacetate (0.35 g, 2.2 mmol) was added dropwise to a solution of LDA [prepared from diisopropyl amine (0.45 g, 4.4 mmol) and *n*-butyl lithium (2.7 mL of a 1.6 mol L^{-1} solution in *n*-hexane, 4.3 mmol) in 20 mL THF] in THF at −10 °C, and the solution was stirred for ten minutes. After cooling to −75 °C, ethyl 2-fluoroacetate (0.21 g, 2.0 mmol) and TMEDA (0.26 g, 2.2 mmol) were added. The solution was stirred for five minutes, warmed to −55 °C over a period of 30 minutes, stirred for an additional ten minutes at this temperature, and then recooled to −75 °C. Workup was carried out as described in the general procedure. After removal of excess *tert*-butyl acetoacetate by Kugelrohr distillation (0.2 mbar, 40 °C), the residue was subjected to flash chromatography on acid-washed silica gel (ethyl acetate/*n*-hexane 10:90 *v*/*v*), and this yielded title compound **1m** as a light-yellow oil. Yield: 0.25 g (58 %).

^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): ratio enol form (ef):keto form (kf) = 90:10; $\delta = 1.47$ (s, $9H_{ef+kf}$; $C(CH_3)_3$), 3.31 (s, $2H_{ef}$; H-2), 3.48 (s, $2H_{kf}$; H-2), 3.86 (d, $^4J(H,F) = 3.8$ Hz, $2H_{kf}$; H-4), 4.86 (d, $^2J(H,F) = 46.8$ Hz, $2H_{ef}$;

H-6), 4.88 (d, ^2J(H,F) = 47.3 Hz, 2 H_{tf}; H-6), 5.93 (d, ^4J(H,F) = 3.0 Hz, 1 H_{ef}; H-4), 14.80 (br s, 1 H_{ef}; OH); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): enol form: δ = 28.1 ($C(CH_3)_3$), 45.9 (C-2), 81.3 (d, ^1J(C,F) = 183.1 Hz, C-6), 82.5 ($C(CH_3)_3$), 97.0 (d, ^3J(C,F) = 5.4 Hz, C-4), 166.5 (C-1), 186.7 (C-3), 189.8 (d, ^2J(C,F) = 21.3 Hz, C-5); MS (70 eV, EI): *m/z* (%): 218 (1) $[M]^+$, 163 (17), 145 (37) $[M - O$*t*$Bu]^+$, 129 (15), 125 (12), 103 (48) $[M - CH_2COO$*t*$Bu]^+$, 57 (100) $[C_4H_9]^+$.

Preparation and assay of recLBADH: A suspension of wet cells (1.0 g) of recombinant *E. coli* strain recADH-HB101 + [9] in phosphate buffer (4.0 mL, 100 mmol L^{-1}, trace of poly(propylene glycol) anti-foam, pH 7.5) was distributed over eight plastic vials (1.5 mL size), and glass beads (8 × 1.2 g, 0.2 mm) were added to each vial. The vials were thoroughly vortexed and cooled in ice. Disruption was carried out in a mixer mill at 0 °C (2 × 5 min with intermediate cooling).[35] Cell debris and glass beads were removed by centrifugation (12000 rpm, 10 min), the supernatants were unified, and $MgCl_2$ was added (final concentration 1.0 mmol L^{-1}). Typical yield: 3 mL, 1100 U mL^{-1}. The enzyme solution thus obtained could be stored for weeks at 4 °C without significant loss of activity. The enzyme activity was determined at 25 °C as follows. A cuvette was charged with a solution (990 µL) of acetophenone (10 mmol L^{-1}) and NADPH (0.25 mmol L^{-1}) in phosphate buffer (100 mmol L^{-1}, $MgCl_2$ 1.0 mmol L^{-1}, pH 6.5), and the reaction was started by addition of enzyme solution [10 µL of a 200-fold dilution in phosphate buffer (100 mmol L^{-1}, $MgCl_2$ 1 mmol L^{-1}, pH 7.5)]. NADPH consumption was monitored at 340 nm (ε_{NADPH} = 6.22 cm^2 µmol^{-1}) over a period of one minute. One unit (U) of activity is defined as the amount of recLBADH that catalyzes the oxidation of 1 µmol NADPH per minute under these conditions. Relative rates for the reduction of β,δ-diketo esters (determined at the concentrations indicated in Table 1) were calculated by setting the rate of acetophenone reduction arbitrarily to 100 %.

recLBADH reduction of β,δ-diketo esters

tert-Butyl (*S*)-*6-chloro-5-hydroxy-3-oxohexanoate* [(*S*)-***2a***]: In a round-bottom flask, a solution of diketo ester **1a** (2.53 g, 10.8 mmol) in 2-propanol (8.3 mL, 108 mmol) was added to phosphate – citrate – NaOH buffer (530 mL, Na_2HPO_4 250 mmol L^{-1}, citric acid 125 mmol L^{-1}, $MgCl_2$ 1 mmol L^{-1}, pH 5.5), and the mixture was vigorously stirred for five minutes. The stirring speed was lowered to 60 rpm, and $NADP^+$ (92 mg, 108 µmol, 90 % purity) and recLBADH (1400 U) were added. After stirring at 20 °C for 14 hours, the mixture was filtered, saturated with NaCl, and extracted with ethyl acetate three times. The unified organic phases were washed with saturated aq. $NaHCO_3$ and brine, dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. Flash chromatography on silica gel (ethyl acetate/isohexane 40:60 *v/v*) gave title compound (*S*)-**2a** as a light-yellow oil. Yield: 1.84 g (72 %).

$[\alpha]_D^{25}$ = −24.9 (*c* = 1.4 in $CHCl_3$); ref. [12]: $[\alpha]_D^{25}$ = −23.0 (*c* = 1.5 in $CHCl_3$), >97 % *ee*; ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): δ = 1.47 (s, 9 H; $C(CH_3)_3$), 2.83 (dd, *J* = 17.5, 7.3 Hz, 1 H; H-4), 2.90 (dd, *J* = 17.5, 5.0 Hz, 1 H; H-4), 3.10 (br s, 1 H; OH), 3.41 (s, 2 H; H-2), 3.57 (dd, *J* = 11.2, 5.0 Hz, 1 H; H-6), 3.62 (dd, *J* = 11.2, 5.1 Hz, 1 H; H-6), 4.31 (m, 1 H; H-5), traces of enol form visible; ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): δ = 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); MS (70 eV, EI): *m/z* (%): 236 (1) $[M]^+$, 163/165 (27/9) $[M - O$*t*$Bu]$, 144 (11), 131 (41), 121/123 (20/6) $[M - CH_2COO$*t*$Bu]^+$, 102 (13), 85 (10), 59 (18), 57 (100) $[C_4H_9]^+$; elemental analysis calcd (%) for $C_{10}H_{17}ClO_4$ (236.7): C 50.74, H 7.24; found: C 50.81, H 7.29.

tert-Butyl (*R*)-*5-hydroxy-3-oxohexanoate* [(*R*)-***2b***]: In a round-bottom flask, a solution of diketo ester **1b** (1.98 g, 9.9 mmol) in 2-propanol (5.1 mL, 66 mmol) was added to 330 mL triethanolamine – HCl buffer (250 mmol L^{-1}, $MgCl_2$ 1 mmol L^{-1}, pH 7.0), and the mixture was vigorously stirred for ten minutes. The stirring speed was lowered to 60 rpm, and $NADP^+$ (28 mg, 33 µmol; 90 % purity) and recLBADH (330 U) were added. After stirring at 20 °C for 24 hours, the solution was saturated with NaCl and extracted with ethyl acetate three times. The unified organic phases were dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. Flash chromatography on silica gel (ethyl acetate/isohexane 40:60 *v/v*) gave title compound (*R*)-**2b** as a colorless oil. Yield: 1.54 g (77 %).

$[\alpha]_D^{25}$ = −40.1 (*c* = 1.9 in $CHCl_3$); ref. [13a]: $[\alpha]_D^{26}$ = −39.6 (*c* = 2 in $CHCl_3$), 99 % *ee*; ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): δ = 1.21 (d, *J* = 6.4 Hz, 3 H; H-6), 1.48 (s, 9 H; $C(CH_3)_3$), 2.64 (dd, *J* = 17.7, 8.5 Hz, 1 H; H-4), 2.74 (dd, *J* = 17.7, 3.2 Hz, 1 H; H-4), 2.91 (br s, 1 H; OH), 3.38 (s, 2 H; H-2), 4.27 (m, 1 H; H-5), traces of enol form visible; ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): δ = 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). The ^{1}H NMR spectrum is in agreement with published data.[13a]

tert-Butyl (*R*)-*5-hydroxy-3-oxoheptanoate* [(*R*)-***2c***]: In a round-bottom flask diketo ester **1c** (150 mg, 0.7 mmol) and 2-propanol (0.5 mL, 6.5 mmol) were added to phosphate buffer (35 mL, 250 mmol L^{-1}, $MgCl_2$ 1 mmol L^{-1}, pH 6.5), and the mixture was vigorously stirred for ten minutes. The stirring speed was lowered to 60 rpm, and $NADP^+$ (15 mg, 18 µmol; 90 % purity) and recLBADH (54 U) were added. The mixture was stirred at 20 °C, and recLBADH (50 U) and $NADP^+$ (10 mg, 13 µmol) were added after 24 and 48 hours each. After a total reaction time of 96 hours, the solution was saturated with NaCl and extracted with ethyl acetate three times. The unified organic phases were washed with aq. $NaHCO_3$ (5 %), water and brine, dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. Flash chromatography on silica gel (ethyl acetate/isohexane 30:70 *v/v*) gave title compound (*R*)-**2c** as a colorless oil. Yield: 92 mg (61 %).

$[\alpha]_D^{25}$ = −36.0 (*c* = 1.0 in $CHCl_3$); ref. [13b]: $[\alpha]_D$ = −35.6 (*c* = 1 in $CHCl_3$), 99 % *ee*; ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): δ = 0.94 (t, *J* = 7.4 Hz, 3 H; H-7), 1.43 – 1.56 (m, 2 H; H-6) overlapping with 1.46 (s, 9 H; $C(CH_3)_3$), 2.61 (dd, *J* = 17.5, 8.9 Hz, 1 H; H-4), 2.73 (dd, *J* = 17.5, 3.0 Hz, 1 H; H-4), 2.90 (br s, 1 H; OH), 3.38 (s, 2 H; H-2), 3.99 (m, 1 H; H-5), traces of enol form visible; ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): δ = 10.0 (C-7), 28.1 ($C(CH_3)_3$), 29.4 (C-6), 49.2, 51.4 (C-2, C-4), 69.0 (C-5), 82.4 ($C(CH_3)_3$), 166.4 (C-1), 204.6 (C-3). The ^{1}H NMR spectrum is in agreement with published data.[13b]

Baker's yeast reduction of diketo ester 1a

tert-Butyl (*R*)-*6-chloro-5-hydroxy-3-oxohexanoate* [(*R*)-***2a***]: Commercially available Amberlite XAD-7 resin was washed prior to use with water, acetone, and ethyl acetate, and dried under reduced pressure until the weight remained constant. Amberlite XAD-7 (9.0 g) was added to a solution of diketo ester **1a** (2.00 g, 8.5 mmol) in ethyl acetate (70 mL), and the solvent was thoroughly evaporated under reduced pressure. The charged resin was added to a suspension of dried Baker's yeast (85 g) in deionized water (400 mL), and the mixture was shaken at 20 °C (130 rpm, 1 L shake flask, horizontal shaker). After 15 hours, the resin was collected on a sintered glass funnel (porosity "0"), washed with a minimal amount of water, and extracted with acetone (4 × 50 mL) and ethyl acetate (50 mL). The extract was concentrated under reduced pressure, and the residue dissolved in ethyl acetate. The solution was washed with aq. $NaHCO_3$ (5 %) and brine, dried over $MgSO_4$, and the solvent evaporated under reduced pressure. Flash chromatography on silica gel (ethyl acetate/isohexane 40:60 *v/v*) gave title compound (*R*)-**2a** as a light-yellow oil. Yield: 1.01 g (50 %).

$[\alpha]_D^{25}$ = +22.8 (*c* = 1.6 in $CHCl_3$); $[\alpha]_D^{25}$ = −24.9 (*c* = 1.4 in $CHCl_3$) for the *S* enantiomer, >99.5 % *ee* (see above). NMR data were in agreement with the data described for (*S*)-**2a**.

Preparation of δ-lactones

(*S*)-*6-Chloromethyl-5,6-dihydropyran-2,4-dione* [(*S*)-***3***]: A catalytic amount of TsOH monohydrate was added to a solution of hydroxy keto ester (*S*)-**2a** (0.24 g, 1.0 mmol) in dichloromethane (15 mL), and the solution was stirred at room temperature for four days. Dichloromethane was replaced by ethyl acetate. The solution was washed with water and brine, dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. Flash chromatography on silica gel (ethyl acetate/diethyl ether/acetic acid 45:55:0.1) gave title compound (*S*)-**3** as a white solid. Yield: 0.11 g (67 %).

$[\alpha]_D^{25}$ = −80.6 (*c* = 1.1 in MeOH); ref. [11a]: $[\alpha]_D^{25}$ = −83.4 (*c* = 1.07 in MeOH), ≥98 % *ee*; ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): δ = 2.72 (dd, *J* = 18.2, 11.1 Hz, 1 H; H-5), 2.89 (dd, *J* = 18.2 Hz, 3.2 Hz, 1 H; H-5), 3.51 (d, *J* = 19.1 Hz, 1 H; H-3), 3.61 (d, *J* = 19.1 Hz, 1 H; H-3), 3.82 (d, *J* = 4.9 Hz, 2 H; CH_2Cl), 4.90 (m, 1 H; H-6). The NMR spectrum is in agreement with data published for the racemate.[11b]

General procedure for the preparation of α,β-unsaturated δ-lactones and determination of the enantiomeric excess

a) *Ketone reduction*: $NaBH_4$ (0.6 equiv) was added portionwise over a period of ten minutes to an ice-cooled solution of the hydroxy keto ester (1 equiv) in ethanol (10 mL per mmol ester), and the mixture was stirred at this temperature for an additional 30 minutes. Acetic acid (2.4 equiv) was added dropwise, and stirring was continued for five minutes. Ethanol was

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replaced by ethyl acetate. The solution was washed with aq. $NaHCO_3$ (5%) and brine, dried over $MgSO_4$, and the solvent was evaporated under reduced pressure.

b) *Lactonization and dehydration*: A catalytic amount of TsOH monohydrate was added to a solution of the crude dihydroxy ester in toluene (10 mL), and the mixture was heated to reflux for two hours. After cooling, ethyl acetate was added. The solution was washed with aq. $NaHCO_3$ (5%) and brine, dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The product was analyzed by HPLC on chiral stationary phase (Daicel Chiracel OB, 250 × 4.6 mm with guard column 50 × 4.6 mm; 25 °C; 1.0 mL min^{-1} isohexane/isopropyl alcohol 80:20 *v*/*v* for **4a**,**b**, 85:15 for **4c**; 215 nm). To verify separation performance, spiking experiments were carried out with *rac*-**4a**–**c**.[36] Retention times: (*S*)-**4a** 24.3 min; (*R*)-**4a** 20.9 min; (*R*)-**4b** 23.8 min; (*S*)-**4b** 17.6 min; (*R*)-**4c** 29.1 min; (*S*)-**4c** 18.7 min.

(*S*)-6-Chloromethyl-5,6-dihydropyran-2-one [(*S*)-4a]: Prepared according to the general procedure from hydroxy keto ester (*S*)-**2a** (270 mg, 1.1 mmol). Flash chromatography on silica gel (ethyl acetate/isohexane 50:50 *v*/*v*) gave title compound (*S*)-**4a** as a light-yellow oil. Yield: 100 mg (60%).

$[\alpha]_D^{25} = -158.6$ ($c = 0.5$ in $CHCl_3$), >99.5% *ee*; ref. [11a]: $[\alpha]_D^{23} = -144.8$ ($c = 3.09$ in $CHCl_3$), ≥98% *ee*; ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): $\delta =$ 2.50–2.65 (m, 2H; H-5), 3.71 (dd, $J = 11.7$, 6.0 Hz, 1H of CH_2Cl), 3.76 (dd, $J = 11.7$, 4.8 Hz, 1H of CH_2Cl), 4.67 (m, 1H; H-6), 6.07 (dt, $J = 9.9$, 1.9 Hz, 1H; H-3), 6.93 (m, 1H; H-4); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): $\delta = 26.9$ (C-5), 44.9 (CH_2Cl), 76.5 (C-6), 121.3 (C-3), 144.7 (C-4), 163.2 (C-2). The ^{1}H NMR spectrum is in agreement with published data.[37]

(*R*)-6-Chloromethyl-5,6-dihydropyran-2-one [(*R*)-4a]: Prepared from hydroxy keto ester (*R*)-**2a** according to the preceding example. HPLC analysis of the crude product indicated 94% *ee*.

(*R*)-6-Methyl-5,6-dihydropyran-2-one [(*R*)-4b]: Prepared according to the general procedure from hydroxy keto ester (*R*)-**2b** (205 mg, 1.0 mmol). The crude product (79 mg, 70%) was not further purified; 99.4% *ee*.

^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): $\delta = 1.37$ (d, $J = 6.4$ Hz, 3H; CH_3), 2.17–2.39 (m, 2H; H-5), 4.51 (m, 1H; H-6), 5.93 (ddd, $J = 9.8$, 2.5, 1.2 Hz, 1H; H-3), 6.83 (ddd, $J = 9.8$, 5.8, 2.7 Hz, 1H; H-4); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): $\delta = 20.7$ (CH_3), 31.0 (C-5), 74.4 (C-6), 121.1 (C-3), 145.3 (C-4), 164.6 (C-2). The ^{1}H NMR spectrum is in agreement with published data.[4b]

(*R*)-6-Ethyl-5,6-dihydropyran-2-one [(*R*)-4c]: Prepared according to the general procedure from hydroxy keto ester (*R*)-**2c** (130 mg, 0.6 mmol). The crude product (49 mg, 65%) was not further purified; 98.1% *ee*.

^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): $\delta = 0.98$ (t, $J = 7.5$ Hz, 3H; CH_3), 1.60–1.85 (m, 2H; CH_2CH_3), 2.20–2.39 (m, 2H; H-5), 4.32 (m, 1H; H-6), 5.96 (ddd, $J = 9.8$, 2.4, 1.4 Hz, 1H; H-3), 6.86 (m, 1H; H-4); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): $\delta = 9.3$ (CH_3), 27.9, 28.9 (C-5, CH_2CH_3), 79.3 (C-6), 121.3 (C-3), 145.4 (C-4), 164.8 (C-2). The ^{1}H NMR spectrum is in agreement with data published for the racemate.[38]

***tert*-Butyl (4-oxo-4,5-dihydrofuran-2-yl)-acetate (5)**: Phosphate buffer (20 mL, 250 mmol L^{-1}, pH 7.0) was added to a solution of diketo ester **1a** (234 mg, 1.0 mmol) in ethanol (10 mL), and the solution was stirred at room temperature for 20 h. Ethanol was removed under reduced pressure. Hydrochloric acid (5 mL, 2 mol L^{-1}) was added, and the mixture was extracted twice with ethyl acetate. The unified organic phases were washed with aq. $NaHCO_3$ (5%) and brine, dried over Na_2SO_4, and the solvent was removed under reduced pressure. Flash chromatography on silica gel (ethyl acetate/isohexane 40:60 *v*/*v*) gave title compound **5** as a light-yellow oil that solidified upon storage at 4 °C. Yield: 157 mg (79%).

^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): $\delta = 1.47$ (s, 9H; $C(CH_3)_3$), 3.48 (s, 2H; $CH_2COOtBu$), 4.53 (s, 2H; H-5), 5.68 (s, 1H; H-3); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): $\delta = 28.1$ ($C(CH_3)_3$), 38.3 ($CH_2COOtBu$), 75.5 (C-5), 82.9 ($C(CH_3)_3$), 106.4 (C-3), 165.9 ($COOtBu$), 187.0 (C-2), 202.7 (C-4); MS (70 eV, EI): *m*/*z* (%): 198 (11) $[M]^+$, 125 (77) $[M - OtBu]^+$, 97 (25), 98 (10), 67 (20), 59 (13), 57 (100) $[C_4H_9]^+$.

Diastereoselective reduction of hydroxy keto ester 2a

tert-Butyl syn-(3R,5S)-6-chloro-3,5-dihydroxyhexanoate [*syn*-(*3R,5S*)-***6a***]: This compound was prepared from crude hydroxy keto ester (*S*)-**2a** (21.5 g, 86 mmol, approximately 95% purity, >99.5% *ee*) according to the procedure described in ref. [12a]. The crude product was obtained in quantitative yield and had a diastereomeric ratio *syn*-**6a**:*anti*-**6a** = 28:1 as was determined at the stage of the acetonide (see below). Stereoisomers with (5*R*) configuration could not be detected. Crystallization from isohexane/ethyl acetate gave title compound *syn*-(3*R*,5*S*)-**6a** as light-yellow crystals (m.p. 50.8–53.0 °C, ref. [12a]: 50–52 °C (hexane/ethyl acetate)). Yield: 12.8 g (62%).

$[\alpha]_D^{25} = -27.0$ ($c = 1.4$ in $CHCl_3$), >99.5% *ee*, diastereomeric ratio *syn*-**6a**:*anti*-**6a** = 205:1; ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): $\delta = 1.45$ (s, 9H; $C(CH_3)_3$), 1.59–1.79 (m, 2H; H-4), 2.42 (d, $J = 6.2$ Hz, 2H; H-2), 3.51 (dd, $J = 11.1$, 5.3 Hz, 1H; H-6), 3.55 (dd, $J = 11.1$, 5.5 Hz, 1H; H-6), 3.71 (brs, 1H; OH), 3.81 (brs, 1H; OH), 4.08 (m, 1H; H-5), 4.24 (m, 1H; H-3); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): $\delta = 28.3$ ($C(CH_3)_3$), 39.5, 42.5, 49.2 (C-2, C-4, C-6), 68.4, 71.6 (C-3, C-5), 81.9 ($C(CH_3)_3$), 172.2 (C-1); MS (70 eV, EI): *m*/*z* (%): 182/184 (9/3) $[M - C_4H_8]^+$, 165/167 (49/16) $[M - OtBu]^+$, 147 (19), 145 (14), 133 (36), 129 (15), 127 (11), 123 (18), 115 (50), 105 (12), 97 (14), 89 (20), 87 (17), 59 (13), 57 (100) $[C_4H_9]^+$, 56 (11); elemental analysis calcd (%) for $C_{10}H_{19}ClO_4$ (238.7): C 50.32, H 8.02; found: C 50.20, H 8.35.

tert-Butyl syn-(3S,5R)-6-chloro-3,5-dihydroxyhexanoate [*syn*-(*3S,5R*)-***6a***]: Prepared from hydroxy keto ester (*R*)-**2a** (2.89 g, 12.2 mmol, 90% *ee*) as described in the preceding example. The crude product was obtained in quantitative yield and had a diastereomeric ratio *syn*-**6a**:*anti*-**6a** = 45:1 as determined at the stage of the acetonide (see below). Crystallization from isohexane/ethyl acetate gave the product (1.51 g, 52%) in the form of orange crystals ($dr = 187:1$, $ee = 98.0\%$). An analytical sample was recrystallized to give the title compound *syn*-(3*S*,5*R*)-**6a** as colorless crystals (m.p. 51.7–52.7 °C).

$[\alpha]_D^{25} = +27.6$ ($c = 1.3$ in $CHCl_3$), >99.5% *ee*, diastereomeric ratio *syn*-**6a**:*anti*-**6a** > 400:1. NMR data were in agreement with the data described for *syn*-(*3R,5S*)-**6a**.

tert-Butyl anti-(3S,5S)-6-chloro-3,5-dihydroxyhexanoate [*anti*-(*3S,5S*)-***6a***]: Hydroxy keto ester (*S*)-**2a** (0.76 g, 3.2 mmol, >99.5% *ee*, dissolved in 5 mL anhydrous acetonitrile) was added dropwise to a solution of tetramethylammonium triacetoxyborohydride (6.74 g, 25.6 mmol) in anhydrous acetonitrile/acetic acid (30 mL, 50:50 *v*/*v*) at −25 °C. After stirring at this temperature for five hours, aq. sodium potassium tartrate (35 mL, 0.5 mol L^{-1}) was added dropwise, and the mixture was warmed to room temperature. Saturated aq. Na_2CO_3 (70 mL) was added, and the mixture was extracted with ethyl acetate four times. The unified organic phases were washed with saturated aq. Na_2CO_3 and brine, dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The crude product was obtained in quantitative yield and had a diastereomeric ratio *anti*-**6a**:*syn*-**6a** = 14:1 as determined at the stage of the acetonide (see below). Crystallization from isohexane/ethyl acetate gave title compound *anti*-(3*S*,5*S*)-**6a** as colorless crystals (m.p. 73.3–75.0 °C). Yield: 0.54 g (70%).

$[\alpha]_D^{25} = +7.2$ ($c = 1.4$ in $CHCl_3$), >99.5% *ee*, diastereomeric ratio *anti*-**6a**:*syn*-**6a** = 211:1; ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): $\delta = 1.46$ (s, 9H; $C(CH_3)_3$), 1.66–1.71 (m, 2H; H-4), 2.42 (d, $J = 6.3$ Hz, 2H; H-2), 3.05 (brs, 1H; OH), 3.60 (brs, 1H; OH) overlapping with 3.53 (dd, $J = 11.0$, 6.5 Hz, 1H; H-6), 3.62 (dd, $J = 11.0$, 5.0 Hz, 1H; H-6), 4.12 (m, 1H; H-5), 4.30 (m, 1H; H-3); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): $\delta = 28.3$ ($C(CH_3)_3$), 39.3, 42.2, 49.6 (C-2, C-4, C-6), 65.5, 68.9 (C-3, C-5), 81.9 ($C(CH_3)_3$), 172.5 (C-1); MS (70 eV, EI): *m*/*z* (%): 182/184 (9/3) $[M - C_4H_8]^+$, 165/167 (48/16) $[M - OtBu]^+$, 147 (16), 145 (11), 133 (30), 129 (15), 127 (11), 123 (19), 115 (45), 105 (10), 97 (13), 89 (21), 87 (12), 59 (12), 57 (100) $[C_4H_9]^+$, 56 (11); elemental analysis calcd (%) for $C_{10}H_{19}ClO_4$ (238.7): C 50.32, H 8.02; found: C 49.92, H 7.95.

tert-Butyl anti-(3R,5R)-6-chloro-3,5-dihydroxyhexanoate [*anti*-(*3R,5R*)-***6a***]: Prepared from hydroxy keto ester (*R*)-**2a** (1.00 g, 4.2 mmol, 94% *ee*) as described in the preceding example. The crude product had a diastereomeric ratio *anti*-**6a**:*syn*-**6a** = 18:1 as determined at the stage of the acetonide (see below). Crystallization from isohexane/ethyl acetate gave title compound *anti*-(3*R*,5*R*)-**6a** as colorless crystals (m.p. 74.5–76.0 °C). Yield: 0.69 g (68%).

$[\alpha]_D^{25} = -6.7$ ($c = 1.3$ in $CHCl_3$), 99.3% *ee*, diastereomeric ratio *anti*-**6a**:*syn*-**6a** = 316:1. NMR data were in agreement with the data described for *anti*-(*3S,5S*)-**6a**.

Determination of the diastereomeric ratio *syn*-6a:*anti*-6a and the enantiomeric excess of dihydroxy ester 6a at the stage of the acetonide 7a: Dihydroxy ester **6a** (10 mg) was dissolved in 2,2-dimethoxy-propane (1 mL), and a catalytic amount of camphorsulfonic acid was added. After shaking at 25 °C for 4–7 h, GC-MS analysis indicated complete conversion,

and the solution was investigated by gas chromatography on chiral stationary phase. Column: CS FS-Cyclodex beta-1/P, 50 m × 0.32 mm ID. Injector: split, 250 °C. Carrier gas: hydrogen, 0.95 bar (constant pressure). Temperature program: 135 °C isothermal. Retention times: *anti*-(3*R*,5*R*)-**7a** 48.8 min; *anti*-(3*S*,5*S*)-**7a** 50.2 min; *syn*-(3*R*,5*S*)-**7a** 52.1 min; *syn*-(3*S*,5*R*)-**7a** 53.8 min. To verify peak indentity and separation performance, spiking experiments were carried out with acetonide *rac*-(3*RS*,5*RS*)-**7a** that was prepared from hydroxy keto ester *rac*-**2a**[36] by sodium borohydride reduction and subsequent acetonide formation.

***tert*-Butyl *syn*-(3*R*,5*S*)-6-chloro-3,5-(isopropylidenedioxy)-hexanoate [*syn*-(3*R*,5*S*)-7a]**: The compound 2,2-dimethoxy-propane (26 mL, 214 mmol) and a catalytic amount camphorsulfonic acid at room temperature were added to a solution of dihydroxy ester *syn*-(3*R*,5*S*)-**6a** (5.10 g, 21.4 mmol, >99.5 % *ee*, *dr syn*-**6a**:*anti*-**6a** = 205:1) in acetone (17 mL). After stirring the solution for 1.5 h, the volatiles were removed under reduced pressure, and the residue was dissolved in ethyl acetate (60 mL). The solution was washed with saturated aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and the solvent was evaporated under reduced pressure, and pure product *syn*-(3*R*,5*S*)-**7a** (5.83 g, 98 %) was left as a colorless oil that solidified upon storage at 4 °C.

$[\alpha]_D^{25} = +3.1$ ($c = 1.3$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): $\delta =$ 1.24 (m, 1H; H-4), 1.39 (s, 3H; CH_3), 1.45 (s, 9H; $C(CH_3)_3$), 1.46 (s, 3H; CH_3), 1.76 (dt, $J = 12.6$, 2.5 Hz, 1H; H-4), 2.33 (dd, $J = 15.2$, 6.1 Hz, 1H; H-2), 2.45 (dd, $J = 15.2$, 7.1 Hz, 1H; H-2), 3.40 (dd, $J = 11.0$, 5.8 Hz, 1H; H-6), 3.51 (dd, $J = 11.0$, 5.6 Hz, 1H; H-6), 4.06 (m, 1H; H-5), 4.28 (m, 1H; H-3); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): $\delta = 19.7$ (CH_3), 28.1 ($C(CH_3)_3$), 29.8 (CH_3), 33.9, 42.5, 47.1 (C-4, C-2, C-6), 65.9, 69.2 (C-3, C-5), 80.7 ($C(CH_3)_3$), 99.2 ($C(CH_3)_2$), 170.0 (C-1); MS (70 eV, EI): *m*/*z* (%): 263/265 (37/12) $[M - CH_3]^+$, 207/209 (70/24), 165 (12), 147/149 (61/18), 129 (18), 115 (11), 111 (12), 105 (17), 59 (53), 57 (100) $[C_4H_9]^+$; HRMS (EI) calcd for $[M - CH_3]^+$: 263.1050; found: 263.1037; elemental analysis calcd (%) for $C_{13}H_{23}ClO_4$ (278.8): C 56.01, H 8.32; found: C 56.06, H 8.33.

***tert*-Butyl *syn*-(3*R*,5*S*)-5,6-epoxy-3-hydroxyhexanoate [*syn*-(3*R*,5*S*)-9]**

Method A: DBU (3.83 g, 25.1 mmol) was added to a solution of dihydroxy ester *syn*-(3*R*,5*S*)-**6a** (3.00 g, 12.6 mmol, >99.5 % *ee*, *dr syn*-**6a**:*anti*-**6a** = 205:1) in dichloromethane (130 mL), and the solution was heated to reflux for 24 hours. The solution was washed with saturated aq. NH_4Cl (2×), saturated aq. $NaHCO_3$ and brine, dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The crude product was obtained as yellow oil that consisted of 87 mol % title compound (3*R*,5*S*)-**9** and 13 mol % tetrahydrofurane (2*R*,4*S*)-**11** according to ^{1}H NMR analysis. Yield: 1.94 g (66 %). An analytical sample was purified by flash chromatography on silica gel (ethyl acetate/isohexane 50:50 *v*/*v*).

$[\alpha]_D^{25} = -30.1$ ($c = 1.7$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): $\delta =$ 1.45 (s, 9H; $C(CH_3)_3$), 1.63–1.78 (m, 2H; H-4), 2.45 (d, $J = 6.2$ Hz, 2H; H-2), 2.50 (dd, $J = 5.0$, 2.7 Hz, 1H; H-6), 2.77 (br t, $J \approx 4.5$–5.0 Hz, 1H; H-6), 3.09 (m, 1H; H-5), 3.33 (d, $J = 3.3$ Hz, 1H; OH), 4.19 (m, 1H; H-3); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): $\delta = 28.3$ ($C(CH_3)_3$), 39.0, 42.1, 46.8 (C-2, C-4, C-6), 49.7 (C-5), 66.4 (C-3), 81.7 ($C(CH_3)_3$), 172.4 (C-1); MS (70 eV, EI): *m*/*z* (%): 146 (5) $[M - C_4H_8]^+$, 145 (14), 129 (21) $[M - O$*t*$Bu]^+$, 127 (18), 111 (21), 110 (13), 89 (20), 87 (49) $[M - CH_2COO$*t*$Bu]^+$, 71 (11), 69 (15), 59 (15), 57 (100) $[C_4H_9]^+$. The more polar tetrahydrofurane (2*R*,4*S*)-**11** was obtained on further eluation as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): $\delta = 1.45$ (s, 9H; $C(CH_3)_3$), 1.74 (ddd, $J = 13.4$, 9.5, 5.5 Hz, 1H; H-3), 2.00 (br s, 1H; OH) overlapping with 2.08 (dd, $J = 13.4$, 5.9 Hz, 1H; H-3), 2.40 (dd, $J = 15.3$, 6.3 Hz, 1H of CH_2COO*t*Bu), 2.55 (dd, $J = 15.3$, 6.8 Hz, 1H of CH_2COO*t*Bu), 3.72 (br d, $J = 9.9$ Hz, 1H; H-5), 4.00 (dd, $J =$ 9.9, 4.5 Hz, 1H; H-5), 4.46–4.53 (m, 2H; H-2 and H-4); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): $\delta = 28.3$ ($C(CH_3)_3$), 41.5, 41.7 (C-3, CH_2COO-*t*Bu), 72.7, 74.6 (C-2, C-4), 75.6 (C-5), 81.0 ($C(CH_3)_3$), 170.6 (C-1); MS (70 eV, EI): *m*/*z* (%): 146 (37) $[M - C_4H_8]^+$, 145 (13), 129 (37) $[M - O$*t*$Bu]^+$, 128 (29), 127 (48), 102 (27), 98 (15), 97 (10), 87 (100) $[M - CH_2COO$*t*$Bu]^+$, 81 (10), 69 (14), 57 (46) $[C_4H_9]^+$; HRMS (EI) calcd for $[M - C_4H_8]^+$: 146.0579; found: 146.0575.

Method B: Finely powdered potassium hydroxide (364 mg, 6.5 mmol) was added to an ice-cooled solution of dihydroxy ester *syn*-(3*R*,5*S*)-**6a** (240 mg, 1.0 mmol, >99.5 % *ee*, diastereomeric ratio *syn*-**6a**:*anti*-**6a** = 205:1) in diethyl ether (10 mL). After stirring at this temperature for one hour, the solution was filtered through a pad of anhydrous $MgSO_4$, and the solvent evaporated under reduced pressure. The crude product was obtained as light-yellow oil that consisted of 90 mol % title compound (3*R*,5*S*)-**9** and 10 mol % tetrahydrofurane (2*R*,4*S*)-**11** according to ^{1}H NMR analysis. Yield: 103 mg (46 %).

***tert*-Butyl *syn*-(3*R*,5*S*)-6-iodo-3,5-(isopropylidenedioxy)-hexanoate [*syn*-(3*R*,5*S*)-10]**

Method A (*halogen exchange*): Acetonide *syn*-(3*R*,5*S*)-**7a** (2.01 g, 7.2 mmol), [18]crown-6 (2.85 g, 10.8 mmol), and finely powdered potassium iodide (23.7 g, 143 mmol) were added to anhydrous *p*-xylene (50 mL), and a stream of nitrogen was introduced for 15 minutes to remove dissolved oxygen. The mixture was vigorously stirred and heated to reflux for three days, whereupon another portion of potassium iodide (5.98 g, 36 mmol) and [18]crown-6 (0.95 g, 3.6 mmol) was added. Heating was continued for 18 hours. Water (50 mL) was added, and the resulting mixture was filtered. The phases were separated, and the aqueous phase was extracted with ethyl acetate. The unified organic phases were washed with aq. $NaHSO_3$ (20 %), saturated aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and volatiles were removed under reduced pressure. Flash chromatography on silica gel (ethyl acetate/isohexane 20:80 *v*/*v*) gave a yellow oil that consisted of title compound (3*R*,5*S*)-**10** (86 mol %) and unreacted acetonide *syn*-(3*R*,5*S*)-**7a** (14 mol %) according to ^{1}H NMR analysis. Yield: 1.61 g (52 %).

^{1}H NMR (300 MHz, $CDCl_3$, 20 °C): $\delta = 1.16$ (dt, $J = 12.5$, 11.5 Hz, 1H; H-4), 1.40 (s, 3H; CH_3), 1.45 (s, 12H; $C(CH_3)_3$) and CH_3), 1.87 (dt, $J = 12.5$, 2.4 Hz, 1H; H-4), 2.33 (dd, $J = 15.1$, 6.1 Hz, 1H; H-2), 2.46 (dd, $J = 15.2$, 7.0 Hz, 1H; H-2), 3.10 (dd, $J = 10.1$, 6.1 Hz, 1H; H-6), 3.17 (dd, $J = 10.1$, 5.7 Hz, 1H; H-6), 3.88 (m, 1H; H-5), 4.27 (m, 1H; H-3); ^{13}C NMR (75.5 MHz, $CDCl_3$, 20 °C): $\delta = 9.4$ (C-6), 19.8 (CH_3), 28.2 ($C(CH_3)_3$), 29.9 (CH_3), 36.3, 42.5 (C-4, C-2), 66.2, 69.1 (C-3, C-5), 80.8 ($C(CH_3)_3$), 99.6 ($C(CH_3)_2$), 170.1 (C-1); MS (70 eV, EI): *m*/*z* (%): 355 (50) $[M - CH_3]^+$, 299 (28), 239 (100), 197 (31), 129 (13), 111 (15), 59 (23), 57 (77) $[C_4H_9]^+$; HRMS (EI) calcd for $[M - CH_3]^+$: 355.0406; found: 355.0397.

Method B (*opening of epoxide*): Epoxide (3*R*,5*S*)-**9** (1.69 g, 7.3 mmol, 87 % purity), anhydrous lithium iodide (3.33 g, 24.9 mmol), and silica gel (2.08 g) were mixed in dichloromethane, and the solvent was evaporated at reduced pressure. The charged silica gel was kept at room temperature for 1 h, extracted with ethyl acetate, and the extract concentrated under reduced pressure. The compound 2,2-dimethoxy-propane (20 mL) and a catalytic amount of camphorsulfonic acid were added, and the solution was stirred at ambient temperature for 24 hours. Volatiles were evaporated under reduced pressure. The residue was dissolved in dichloromethane, and the solution was washed with aq. $Na_2S_2O_3$ (5 %), saturated aq. $NaHCO_3$, and brine. After drying over Na_2SO_4, the solvent was evaporated under reduced pressure. Flash chromatography on silica gel (ethyl acetate/isohexane 20:80 *v*/*v*) gave title compound (3*R*,5*S*)-**10** as a yellow oil. Yield: 1.55 g (58 %). $[\alpha]_D^{25} = +11.2$ ($c = 2.3$ in $CHCl_3$). NMR data were in agreement with the data of the major product obtained by method A.

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