Enzymatic Kinetic Resolution of 1,3-Dioxolan-4-one and 1,3-Oxathiolan-5-one Derivatives: Synthesis of the Key Intermediate in the Industrial Synthesis of the Nucleoside Reverse Transcriptase Inhibitor AMDOXOVIR

Alfred Popp,[a,*] Andrea Gilch,[a] Anne-Laure Mersier,[a] Hermann Petersen,[b] Jodoca Rockinger-Mechlem,[a] Jürgen Stohrer[a]

[a] Consortium für elektrochemische Industrie GmbH, Zielstattstrasse 20, 81379 München, Germany
Fax: (+49)-89-74844-242, e-mail: alfred.popp@wacker.com
[b] Wacker-Chemie GmbH, Johannes-Hess-Strasse 24, 84489 Burghausen, Germany

Received: October 21, 2003; Accepted: March 26, 2004

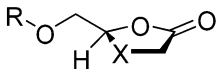
Abstract: The resolution of racemic 1,3-dioxolan-4-one and 1,3-oxathiolan-5-one derivatives such as (4-oxo-1,3-dioxolan-2-yl)methyl 2-methylpropanoate (**2**) by enzymatic solvolysis was investigated. The resolution of **2**, a precursor for the synthesis of the nucleoside reverse transcriptase inhibitor Amdoxovir, was optimized in terms of solvent/nucleophile, reaction conditions, and enzyme. The use of lipase from *Candida antarctica B* (CALB) and methanol as nucleophile and solvent resulted in an effective resolution and the product **(*R*)-2** could be easily isolated. Products of substrate decomposition can be isolated and reused for the synthesis of racemic **2**. The broad range of application for this enzymatic resolution was demonstrated by the resolution of further substrates with different substitution patterns. This process gives a new and unprecedented access to enantiopure 1,3-dioxolan-4-ones and 1,3-oxathiolan-5-ones.

Keywords: enzyme catalysis; kinetic resolution; lipases; solvolysis

Introduction

Enantiopure compounds are used as starting materials and intermediates in the synthesis of agrochemicals and pharmaceuticals. Many of these compounds are currently prepared and marketed as racemates or mixtures of diastereomers. However, in many cases, the desired physiological effect is brought about by only one enantiomer/diastereomer. The other isomer is, in the most favorable case, inactive, but it also may counteract the desired effect or even be toxic. Besides from stereoselective processes, the resolution of racemates is of great importance for the preparation of compounds of high enantiopurity.

Especially enantiopure dioxolan-4-one (X = O) and oxathiolan-5-one derivatives (X = S, Scheme 1) are of great interest because they are intermediates in the synthesis of compounds active in anti-viral therapy. For example, 2-(hydroxymethyl)-1,3-dioxolan-4-one (Scheme 1: X = O, R = H, TBDPS) is a key intermediate in the synthesis of Dioxolane-T,[1,2] whereas 2-(hydroxymethyl)-1,3-oxathiolan-5-ones (Scheme 1: X = S, R = alkyl, silyl, acyl) can be used as building blocks for the preparation of the oxathiolanyl-nucleoside Coviracil®.[2–4]

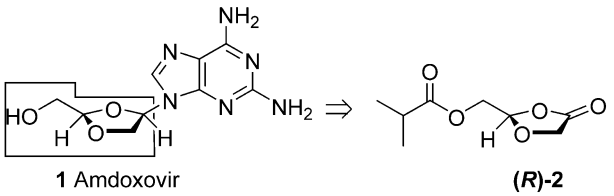


Scheme 1. Enantiopure dioxolan-4-one and oxathiolan-5-one derivatives.

Due to its chemical nature this structure is not prone to resolution by crystallization of diastereomeric salts. To date, no direct resolution of the mentioned racemates has been reported. Only the indirect route *via* enzymatic cleavage of hydrolyzable groups in the side chain in the 2-position of the ring has been described.[1,3–6]

Amdoxovir (**1**) {(−)-(2*R*,4*R*)-2-amino-[2-(hydroxymethyl)-1,3-dioxolan-4-yl]adenine also known as (−)-β-D-2,6-**dia**mino**p**urine **d**ioxolane or DAPD}, originally developed at the University of Georgia,[7] Emory University[8] and Triangle Pharmaceuticals Inc.,[9–11] is an experimental nucleoside reverse transcriptase inhibitor (NRTI)[12] now owned by Gilead Sciences, Inc.[13] As Amdoxovir is currently being evaluated in phase II, it was necessary to develop an economic and reliable industrial large-scale process[14] in order to fulfill the material requirements for clinical trials. In this process, according to retrosynthetic studies the most important key intermediate is the enantiopure 1,3-dioxolan-4-one **(*R*)-2** (Scheme 2).

  DOI: 10.1002/adsc.200303184



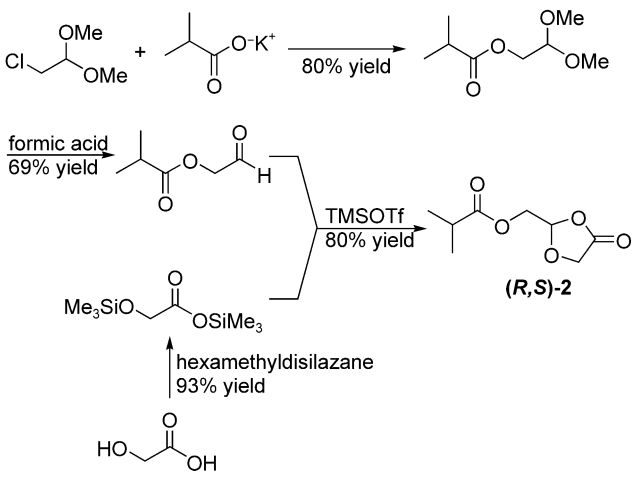
Scheme 2. Retrosynthetic identification of the key building block.

Results and Discussion

Resolution strategies have always played a central role in the preparation of optically active compounds despite the fact that the maximum theoretical yield is 50% based on the racemic starting material. Considering this fact, the basis of an economically successful resolution process is a cheap racemate. Even if the unwanted enantiomer (distomer[15]) can be returned to the production process (e.g., after racemization) this involves additional work and occupancy of reactors. In our case the racemic starting material **(*R,S*)-2** can be obtained according to known protocols.[16]

Starting from chloroacetaldehyde dimethyl acetal and potassium butyrate, a nucleophilic substitution leads to the acetal-protected 2-methylpropanoate which can be deprotected in a second step affording 2-oxoethyl 2-methylpropanoate (**6**).[17] Together with di-silylated[18] glycolic acid (**7**) and trimethylsilyloxy trifluoromethanesulfonate (TMSOTf) as a catalyst the racemic dioxolanone **(*R,S*)-2** can be formed in good yields which can be easily purified by distillation (Scheme 3).[19–21]

This pathway affords an easy and cheap access to the desired racemate on a multi-ton scale without having to resort to elaborate purification steps. The main impurity 2-oxoethyl 2-methylpropanoate (**6**) does not disturb further process steps because it is also formed during the resolution process (*vide supra*).



Scheme 3. Synthesis of **(*R,S*)-2**.

Enzymatic Solvolysis of (R,S)-2

Hydrolysis

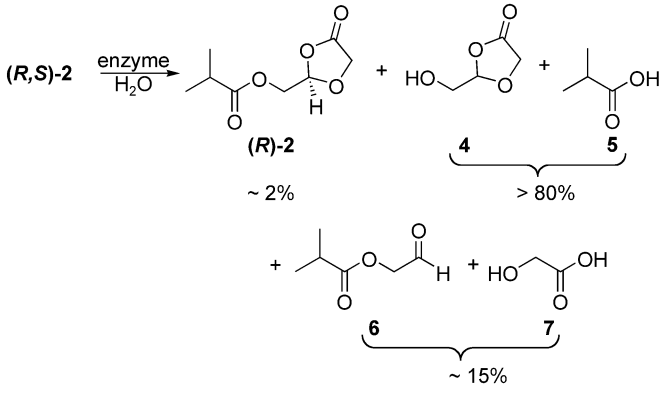
One of the most common methods for resolving racemates of esters and similar compounds is the enzyme-catalyzed aqueous hydrolysis.[22–24] The major drawback of this method is the insufficient solubility of most substrates in aqueous solutions, but this can be avoided by adding organic solvents. In order to identify an appropriate enzyme, we firstly used a two-phase system [0.1 M phosphate puffer at pH 7 and *tert*-butyl methyl ether (TBME)] and 30 commercially available lipases and esterases.[25]

Since, during a hydrolysis, both ester and lactone moieties of **(*R,S*)-2** can be cleaved several degradation products are possible (Scheme 4).

The hydroxymethyldioxolanone **4** and *iso*-butyric acid **5** are formed after hydrolysis of the ester group in the side-chain whereas **6** and **7** are fragments of the cleavage of the dioxolanone ring. We could identify all these products during our screening of the various lipases (except for the stereochemistry of the byproduct **4**), but in most cases a rapid and uncontrollable hydrolysis of the side-chain ester moiety took place. An enrichment of one enantiomer in the remaining substrate was observed with several enzymes but the enantiomer ratio[26] that we obtained was rather low ($E = 2-3$). Neither variation of the amount of water, of pH or buffer strength nor the variation of the organic co-solvent showed any benefit. The reason for this inferior selectivity is the lack of stability of the racemate in the presence of water. Further investigations showed that, depending on the pH, especially the side-chain ester moiety of **(*R,S*)-2** hydrolyzes readily in the absence of enzymes. The addition of enzymes enhances this ester hydrolysis.

Alcoholysis

The experience from hydrolysis experiments indicated that, in order to suppress the unwanted non-enzymatic



Scheme 4. Enzymatic hydrolysis of **(*R,S*)-2**.

reaction, the reactant (also serving as a solvent) should be a weaker nucleophile. Therefore, we screened lower alcohols as nucleophiles [R = Me, Et, *i*-Pr, *n*-Pr, $(CH_2)_7CH_3$]. In this screening, alcoholysis showed a similar spectrum of products as hydrolysis (Scheme 5).

Formation of the metastable hemiacetal **8** was verified with NMR methods. During the reaction ($T < 50\,^{\circ}C$) almost no aldehyde **6** could be detected. Instead of this, three significant NMR signals [$\delta = 4.72$ (dd, $J_1 = 4.9$ Hz, $J_2 = 4.7$ Hz, 1H, COO-CH_2-C<u>H</u>), 4.09 (dd, $J_1 = 11.4$, $J_2 = 4.7$ Hz, 1H, COO-C<u>H_2</u>), 4.05 (dd, $J_1 = 11.4$, 4.9 Hz, 1H, COO-C<u>H_2</u>)] imply the formation of **8**. After warming the reaction mixture (e.g., removing the solvent) these signals were missing. Instead the NMR signals of aldehyde **6** were observed. So a quantitative monitoring of the reaction turnover is possible.[27] The absolute stereochemistry of the product was established by comparing the sign of the optical rotation with a sample that was further processed to give the known compound **1**.

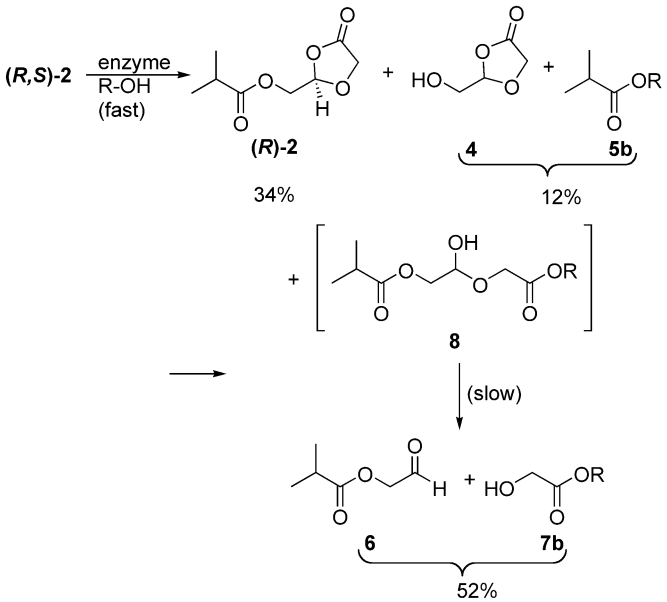
Contrary to the behavior of ***(R,S)*-2** in water, the racemate is chemically reasonably stable in lower alcohols (e.g., MeOH, EtOH). Therefore, the next step was identification of an appropriate enzyme and alcohol. Enzyme screening was carried out using a binary mixture of alcohol and co-solvent [ROH/TBME = 1:1 (v/v)]. Under these conditions we identified two enzymes which catalyzed the desired reaction with a reasonable selectivity: pig pancreas lipase (PPL) and *Candida antarctica* lipase B (CALB). Further studies revealed that the enzyme of choice was CALB together with methanol as nucleophile.

There are two formulations of CALB available from Novozymes A/S (Bagsvaerd, Denmark) on a large scale, Novozym 525 L (NZ525L) which is a rather dilute solution of the enzyme in water and Novozym 435 (NZ435) which contains the enzyme in an immobilized form. Although both formulations show an identical *E* value of 12 (Figure 1) we chose the immobilized formulation because there is only a minimum amount of water in the reaction mixture (water coming from the enzyme formulation) and the reaction mixture can easily and quickly be separated from the catalyst after the reaction.

The fast separation of the catalyst, as mentioned above, is of vital importance because, with an enantiomeric ratio of 12, an appreciable amount of the wanted enantiomer is also converted. This means that, after the wanted enantiomeric excess is reached, any subsequent reaction reduces the yield.

Enzymes normally show their best activity and selectivity at an optimal temperature. Low temperatures slow down the rate of the enzymatic reaction whereas the enantiomeric ratio is only slightly increased (lowering the temperature by 10 K results in an increase: $E_{low\ temp.} \sim E_{high\ temp.} + 1$[28]). The temperature optimum was determined in the temperature range between 20 and 40 °C. The resulting *E* values (Figure 2) and reaction rates (Figure 3) are approximately (within the error limits) independent of the temperature (Table 1). The initial enzyme activity also seemed to be constant within the selected temperature range. These findings indicate a stable process in that the enzyme tolerates small fluctuations in temperature which often occur in industrial processes.

All scale-up experiments (*vide supra*) were carried out at the initial substrate concentration of 15% (w/v) (approx. 0.65 $mol \cdot L^{-1}$). With regard to a high space-time yield, however, the substrate concentration had to be increased (and optimized). A screening of several concentrations revealed no significant change in the enantiomeric ratio up to a concentration of 40% (w/v) (Fig-



Scheme 5. Enzymatic alcoholysis of ***(R,S)*-2**.

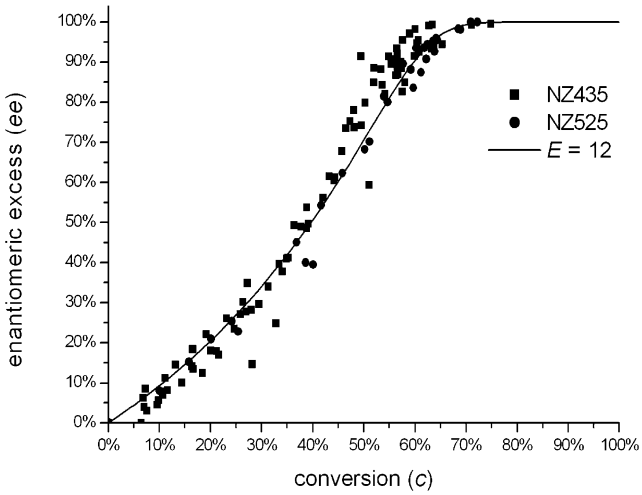


Figure 1. Selectivity of the resolution of ***(R,S)*-2**.

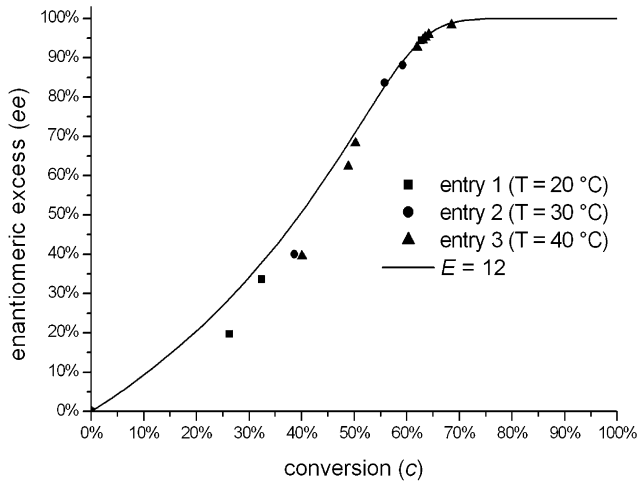


Figure 2. Temperature influence on the enantiomeric ratio of the resolution of ***(R,S)*-2**.

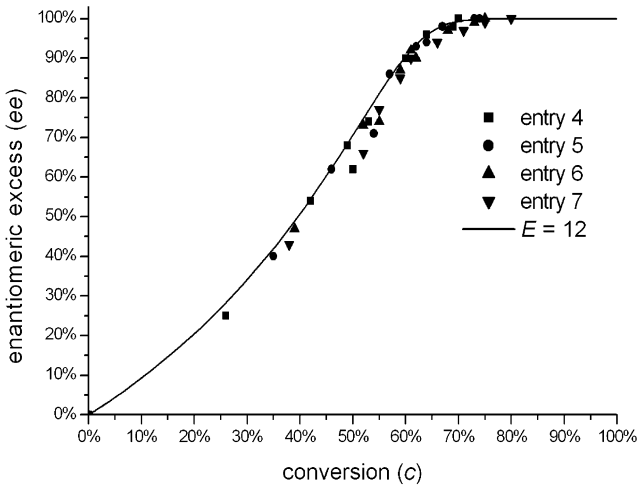


Figure 4. Dependence of the enantiomeric ratio on the substrate concentration (see also Table 2: entries 4–7).

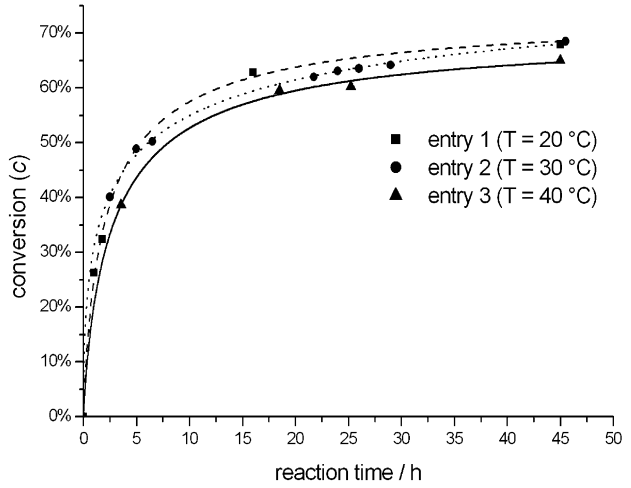


Figure 3. Temperature influence on the reaction rate of the resolution of ***(R,S)*-2**.

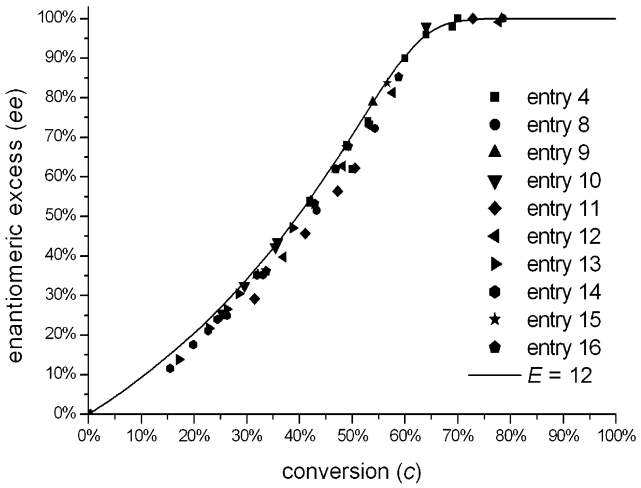


Figure 5. Dependence of the enantiomeric ratio on the substrate concentration (see also Table 2: entries 8–16).

ure 4). In addition, when using NZ525L, the reaction rate is constant (Table 2: entries 4–7).

We evaluated the tolerance of the enzyme towards varying solvent compositions and observed very little influence on the process (Table 2). Small fluctuations of the composition, which often appear in industrial processes after the solvent recycling, have no significant impact either on the selectivity or on the relative activity of the enzyme (entries 8, 9, 11 and 12). In contrast, the relative activity of the enzyme declines when using an excess of methanol (entries 10 and 14). This effect can be compensated for by using a higher enzyme concentration (e.g., doubling of the concentration: entries 13/14 *vs.* 15/16). Nevertheless, the enzyme does not completely loose its activity, even in pure methanol.

After we had established an optimized and robust process a suitable work-up procedure was developed. Actually, the easiest way to purify the product by chromatographic separation of the degradation products. This worked very well on a laboratory scale but could not be adapted to a large-scale production. The method of choice for the purification of **(*R*)-2** proved to be as follows. After the removal of the solvents, the low-boiling degradation products **5b**, **7b** and the main fraction of **6** by distillation, the separation of the hydroxymethyldioxolanone **4** proved to be critical. During the resolution compound **4** was formed at about 20% (w/w) corresponding to the amount of product.[30] In addition to this, **4** could not be separated by distillation because its boiling point differs only slightly from that of the product **(*R*)-2**. However, the water solubility of the primary alcohol **4** is significantly higher than that of the dioxolanone. The byproduct could thus be completely removed by multiple or continuous extractions with water. We favor the continuous extraction method, be-

Table 1. Temperature influence on the reaction rate.[a]

Entry	Reaction temperature [°C]	Conversion [%] after 20 h	Initial enzyme activity[b] [μmol·min^{-1}·g^{-1}]
1	20	64	440
2	30	61	400
3	40	59	410

[a] **(*R*,*S*)-2** (0.65 mol·L^{-1}), naphthalene (0.7 mol equiv. as internal standard) in TBME/MeOH 1:1 (v/v), NZ525L (6.1 g·L^{-1}).
[b] The activity was calculated using following equation[29]: $initial\ activity = \Delta n_{substrate} \cdot \Delta t^{-1} \cdot m_{enzyme\ formulation}{}^{-1}$.

Table 2. Dependence of the reaction rate on the substrate concentration and the solvent composition.

Entry	Substrate concentration[a] [%] (w/v)	Composition of TBME/MeOH (v/v)	Enzyme concentration[b] [g·L^{-1}]	Initial enzyme activity[c] [μmol·min^{-1}·g^{-1}]
4	15	1:1	6.1	440
5	20	1:1	8.2	430
6	30	1:1	12.3	420
7	40	1:1	16.4	440
8	15	2:1	6.1	400
9	15	3:2	6.1	400
10	15	1:2	6.1	280
11	40	2:1	16.4	420
12	40	3:2	16.4	440
13	40	2:9	9.5	430
14	40	1:9	10.0	360
15	40	2:9	19.0	520
16	40	1:9	20.0	490

[a] Naphthalene (0.3 mol-equiv. as internal standard) at 20 °C.
[b] NZ525L.
[c] See footnote[b] in Table 1.

cause it is fast [having in mind the poor stability of **(*R*,*S*)-2** in aqueous media] and can be scaled up easily. The final purification of the product proceeded by vacuum distillation. The forerun contained mainly the residual aldehyde **6** which could be reused (together with the amount separated in advance) for the preparation of **(*R*,*S*)-2** (Scheme 3).

Using a loop reactor and the immobilized formulation of CALB (NZ435), the scale-up proved to be fairly easy and economic. The optimized reaction parameters [**(*R*,*S*)-2** (0.65 mol·L^{-1}) in TBME/MeOH 1:1 (v/v), NZ435 (8.5 g·L^{-1}), 25±5 °C, average retention time in the reactor loop: 1.7 min] were transferred to a pilot-plant scale [10 kg **(*R*,*S*)-2**/batch]. Neither the *E* value nor the activity of the enzyme changed. The resolution of **2** on a 1000-kg scale (seven batches overall) also displayed the same performance.

Enzymatic Solvolysis of 1,3-Dioxolan-4-one and 1,3-Oxathiolan-5-one Derivatives

In order to demonstrate the broad applicability we applied the before-mentioned resolution to chemically similar substrates. The following examples are to evaluate the scope of the resolution process and to perform a preliminary structure-activity relationship study.

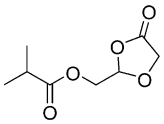
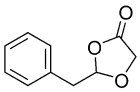
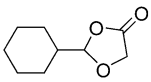
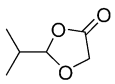
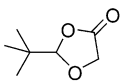
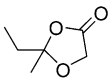
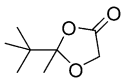
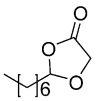
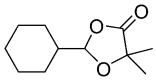
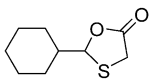
All racemates were synthesized from di-silylated glycolic acid and the appropriate aldehyde according to the procedure described above in good to excellent yields. For known compounds (see references in Table 3) the analytical data were identical to literature data. Then we studied the suitability of the obtained racemates for an enzymatic resolution. A preliminary screening showed that the enzyme of choice was CALB [either immobilized (NZ435) or as crude solution (NZ525L)]. With this enzyme moderate to good enantiomeric ratios could be reached at reasonable enzyme activities (Table 3). Not surprisingly, we observed that the *E* value and the activity depended strongly on the structure of the substrate. From these substrates we derived the following structural requirements for the enzymatic resolution of dioxolanone derivatives:

– The 2-position of the dioxolanone ring bears at least one hydrogen atom. The experiments show that a second substituent at the stereogenic center prevents the conversion (compounds **13** and **14**). This might be due

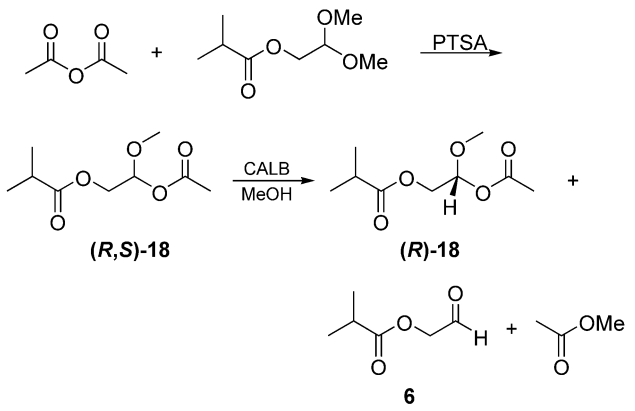
to a strong steric interaction between the enzyme and the substrate that blocks the access to the active site. But what is more important, both compounds are cyclic esters of tertiary alcohols. It is known that lipases solvolyse such esters only in rare cases.
- The substrate must not be disubstituted at the 5-position of the dioxolanone ring (compound **16**).
- Sterically demanding rigid substituents in the 2-position of the dioxolanone ring increase the selectivity of the resolution (compound **10** *vs.* **9**, **11** and **15**). But the α-carbon of the side-chain must not be quaternary (compound **12**) likely because of unfavorable steric interactions.
- If one oxygen atom is replaced by a sulfur atom (compound **17**) the reaction still proceeds with a high *E* value (compound **17** *vs.* **10**). During this resolution, however, we observed a significant decrease of the reaction rate. This is due to an unfavorable equilibrium position. The reaction even stops after the conversion has reached approximately 45% and an ee of the remaining enantiomer of 80%. After removing the degradation products and purifying the enantiomerically enriched substrate the subsequent resolution proceeds smoothly and we obtained an enantiopure product oxathiolan **17**.

The described method for the resolution of dioxolanones can also be expanded to acyclic compounds. Until now this procedure is still limited to stable hemi-acylals, but further work will be done to investigate the limits of the resolution process and to collect more data for a reliable SAR study. With our method the enantiopure hemi-acylal **(*R*)-18** was prepared in good yields (Scheme 6).[31]

Table 3. Enzymatic resolution of dioxolanone- and oxathiolan-derivatives compared to compound **2**.[a]

Compound		*E*	Initial enzyme activity[d] [μmol·min^{-1}·g^{-1}]	$[\alpha]_D^{20}$	ee [%]
2		12	970	+19.8 (d = 1.18, neat)	>98[b]
9[29]		11	680	+0.7 (d = 1.19, neat)	96[b]
10[20]		65	1380	+3.9 (d = 1.11, neat)	90[b]
11[30]		14	370	+1.9 (d = 1.13, neat)	98[b]
12[31]		n.d.	30	n.d.	
13		n.d.	0	n.d.	
14[19]		n.d.	0	n.d.	
15		13	850	+5.5 (d = 1.11, neat)	>99[c]
16		n.d.	10	n.d.	
17		65	100	+6.1 (d = 1.19, neat)	96[b]

[a] Substrate concentration [15% (w/v)], naphthalene (0.4 mol-equiv. as internal standard) in TBME/MeOH 1:1 (v/v), NZ435 (2.5 g·L^{-1}).
[b] Determined by GC method.
[c] Determined by HPLC method.
[d] See footnote[b] in Table 1.



Scheme 6. Preparation of the racemic hemi-acylal **(*R,S*)-18** and subsequent enzymatic alcoholysis.

The resolution proceeded with an *E* value of 15, comparable to that of the non-rigid dioxolanones and an initial enzyme activity of 120 $\mu mol \cdot min^{-1} \cdot g^{-1}$ and which is significantly lower than that of its corresponding cyclic substrate **2** (Table 3).

Conclusions

Developing an enzymatic resolution process of dioxolanones and related compounds we have established an easy and economic method for the preparation of important enantiopure synthetic precursors of pharmaceuticals. The development of an industrial resolution of **2** shows in an impressive manner the power of enzymatic resolutions for the production of pharmaceutical key intermediates (e.g., Amdoxovir). The pilot process gave the pure product **(*R*)-2** (1100 kg with ee > 98%) with an overall yield of 22% [enzyme load: NZ435/**(*R,S*)-2** = 3% (w/w)].

We also synthesized a variety of (a)cyclic racemates and employed them in our enzymatic resolution protocol. Our findings are the basis for future structure-activity relationship (SAR) studies to further elaborate on the presented substrate requirement rules.

Experimental Section

Spectroscopic Methods

^{1}H NMR spectra were recorded in deuterochloroform with TMS as internal reference on a Bruker Avance 500 spectrometer operating at 400 MHz. Enantiomeric excesses were either determined by gas chromatography (HP5890II gas chromatograph equipped with an FID detector; column: CP Chirasil CB; injector temperature: 220 °C; detector temperature: 250 °C) or HPLC [HP 1100 HPLC equipped with an UV detector (200, 204 und 210 nm); column: Chiralpak AD-RH; isocratic operation].

Procedure for the Preparation of the Dioxolanone Derivatives 2–16

Compounds known from literature were also prepared in accordance with this method.

A stirred solution of di-silylated glycolic acid (**7**; 0.40 mol) and Me_3SiOTf (12.0 mmol) in anhydrous dichloromethane (250 mL) was cooled to −78 °C. To this solution the appropriate aldehyde (0.44 mol) was added dropwise. After warming to room temperature the solution was stirred for further 20 h at this temperature. The reaction mixture was washed with saturated aqueous sodium bicarbonate and dried over sodium sulfate. The solvent was removed under vacuum and the residue was purified by distillation to give the desired products.

Isobutyric acid 4-oxo-[1,3]dioxolan-2-ylmethyl ester (**2**) using (**6**): colorless liquid, bp 55–58 °C/0.02 mbar; ^{1}H NMR: δ = 5.80 (dd, J_1 = 2.8 Hz, J_2 = 3.2 Hz, 1H, $CHCO_2$), 4.37 (dd, J_1 = 2.8 Hz, J_2 = 12.4 Hz, 1H, COO-CH_2), 4.26 (dd, J_1 = 3.2 Hz, J_2 = 12.4 Hz, 1H, COO-CH_2), 4.38 (dd, J_1 = 15.0 Hz, J_2 = 0.8 Hz, 1H, OCH_2CO), 4.29 (dd, J_1 = 15.0 Hz, J_2 = 0.6 Hz, 1H, OCH_2CO), 2.60 [sept, 1H, C$\underline{H}$(CH_3)$_2$], 1.15 [d, 6H, CH(C$\underline{H_3}$)$_2$]; $t_{r,GC}$ (min) = 19.4 (*R*), 19.7 (*S*).

2-Ethyl-2-methyl-[1,3]dioxolan-4-one (**13**) using butan-2-one: colorless liquid, bp 140–142 °C; ^{1}H NMR: δ = 4.37 (d, *J* = 15.3 Hz, 1H), 4.32 (d, *J* = 15.3 Hz, 1H), 1.85 (q, *J* = 7.5 Hz, 2H), 1.50 (s, 3H), 1.00 (t, *J* = 7.5 Hz, 3H); $t_{r,HPLC}$ (min) = 6.6 (*R*), 7.1 (*S*).

2-Heptyl-[1,3]dioxolan-4-one (**15**) using octanal: colorless liquid, bp 90–92 °C/7 mbar; ^{1}H NMR: δ = 5.60 (t, *J* = 4.9 Hz, 1H), 4.35 (d, *J* = 15.0 Hz, 1H), 4.20 (d, *J* = 15.0 Hz, 1H), 1.85 (m, 2H), 1.40 (m, 2H), 1.25–0.85 (m, 11H); $t_{r,GC}$ (min) = 21.7 (*R*), 22.0 (*S*).

2-Cyclohexyl-5,5-dimethyl-[1,3]dioxolan-4-one (**16**) using cyclohexylcarbaldehyde: colorless liquid, bp 104–106 °C/0.7 mbar; ^{1}H NMR: δ = 5.25 (d, *J* = 4.6 Hz, 1H), 1.90 (m, 4H), 1.80 (m, 2H), 1.50 (s, 3H), 1.40 (s, 3H), 1.30–1.10 (m, 5H). $t_{r,GC}$ (min) = 21.7 (*R*), 22.1 (*S*).

2-Cyclohexyl-[1,3]oxathiolan-5-one (**17**): A solution of mercaptoacetic acid (0.42 mol, 38.6 g) in anhydrous dichloromethane (250 mL) was cooled to 0 °C. Cyclohexylcarbaldehyde (0.42 mol, 49.2 mL) was added dropwise, the resulting solution was brought to room temperature and stirred for 20 h at this temperature. The reaction mixture was washed with saturated aqueous sodium bicarbonate and dried over sodium sulfate. The solvent was removed under vacuum and the residue was purified by distillation to give the pure compound as a colorless liquid; bp 120–124 °C/5 mbar; ^{1}H NMR: δ = 5.30 (d, *J* = 6.5 Hz, 1H), 3.65 (d, *J* = 16.6 Hz, 1H), 3.60 (d, *J* = 16.6 Hz, 1H), 2.00 (m, 1H), 1.80 (m, 4H), 1.45–1.15 (m, 6H); $t_{r,GC}$ (min) = 23.7 (*R*), 24.2 (*S*).

2-(Acetyloxy)-2-methoxyethyl 2-methylpropanoate (**18**): *p*-Toluenesulfonic acid monohydrate (PTSA; 3.90 mmol, 0.75 g) was dissolved in a mixture of 2,2-dimethoxyethyl 2-methylpropanoate (0.28 mol, 50.0 g) and acetic anhydride (0.34 mol, 34.8 g). The solution was heated to 120 °C for 12.5 h. After cooling to room temperature the reaction mixture was washed with H_2O (2 × 50 mL) and dried over sodium sulfate. After filtration the residue was purified by distillation to give the pure compound as a colorless liquid; yield: 37.6 g (0.18 mol, 63%); bp 52–55 °C/0.3 mbar; ^{1}H NMR: δ = 5.91 (dd, J_1 = 5.4 Hz, J_2 = 4.9 Hz, 1H), 4.24 (dd, J_1 = 11.6, J_2 = 4.9 Hz, 1H), 4.07 (dd, J_1 = 11.6, J_2 = 5.4 Hz, 1H), 3.48 (s, 3H),

2.58 (sept, $J = 7.0$, 1H), 2.12 (s, 3H), 1.17 (d, $J = 7.0$ Hz, 6H); $t_{r,GC}$ (min) = 17.7 (*R*), 18.6 (*S*).

Typical Procedure for the Preparation of (*R*)-(+)-Isobutyric Acid 4-Oxo-[1,3]dioxolan-2-ylmethyl Ester (2)

All enzymatic resolutions were performed according to this method.

(*R,S*)-2 (50.0 g, 0.27 mol) was dissolved in a mixture of TBME (185 mL) and MeOH (185 mL) in a 1-L 4-necked flask. Novozym 435 (NZ435, 2.6 g) was added and the resulting mixture was stirred vigorously at 30 °C. After the desired ee was reached (GC control) the enzyme was filtered off and the solvent was removed under reduced pressure. The residue was then taken up in TBME (100 mL) and washed twice with water (100 mL each). The organic phase was separated, dried over sodium sulfate and then freed from solvent under vacuum. The crude product was purified by distillation to give the product **(*R*)-2** as a colorless liquid; overall yield: 10.3 g (0.05 mol, 20%).

References and Notes

[1] L. J. Wilson, W. B. Choi, T. Spurling, D. C. Liotta, R. F. Schinazi, D. Cannon, G. R. Painter, M. St. Clair, P. A. Furman, *Bioorg. Med. Chem. Lett.* **1993**, *3*, 169–174.

[2] W. B. Choi, L. J. Wilson, S. Yeola, D. C. Lotta, R. F. Schinazi, *J. Am. Chem. Soc.* **1991**, *113*, 9377–9379.

[3] D. C. Lotta, R. F. Schinazi, W. B. Choi, (Emory University, USA), *WO* 9214743 A2, **1992**; *Chem. Abstr.* **1993**, *118*, 22551.

[4] D. C. Liotta, W. B. Choi, (Emory University, USA), *WO* 9111186 A1, **1991**; *Chem. Abstr.* **1991**, *115*, 208463.

[5] L. K. Hoong, L. E. Strange, D. C. Liotta, G. W. Koszalka, C. L. Burns, R. F. Schinazi, *J. Org. Chem.* **1992**, *57*, 5563–5565.

[6] Y. Yao, Y. F. Wang, (Altus Biologics Inc., USA), *WO* 2000022157 A1, **2000**; *Chem. Abstr.* **2000**, *132*, 278245.

[7] H. O. Kim, R. F. Schinazi, S. Nampalli, K. Shanmuganathan, D. L. Cannon, A. J. Alves, L. S. Jeong, J. W. Beach, C. K. Chu, *J. Med. Chem.* **1993**, *36*, 30–37.

[8] A. H. Corbett, J. C. Rublein, *Current Opinion in Investigational Drugs* **2001**, *2*, 348–353.

[9] P. A. Furman, D. Cleary, L. C. Trost, J. W. Bigley, G. R. Painter, *Drugs Future* **2000**, *25*, 454–461.

[10] P. A. Furman, J. Jeffrey, L. L. Kiefer, J. Y. Feng, K. S. Anderson, K. Borroto-Esoda, E. Hill, W. C. Copeland, C. K. Chu, J.-P. Sommadossi, I. Liberman, R. F. Schinazi, G. R. Painter, *Antimicrobial Agents and Chemotherapy* **2001**, *45*, 158–165.

[11] P. A. Furman, G. R. Painter, D. Barry, F. Rousseau, (Triangle Pharmaceuticals, Inc., USA), *WO* 2000025797 A1, **2000**; *Chem. Abstr.* **2000**, *132*, 318062.

[12] Z. Gu, M. A. Wainberg, N. Nguyen-Ba, L. L'Heureux, J.-M. De Muys, T. L. Bowlin, R. F. Rando, *Antimicrob. Agents Chemother.* **1999**, *43*, 2376–2382.

[13] L. K. Naeger, N. A. Margot, M. D. Miller, *Antimicrob. Agents Chemother.* **2003**, *46*, 2179–2184.

[14] A. Popp, J. Stohrer, H. Petersen, A. Gilch, J. Rockinger-Mechlem, (Consortium fuer elektrochemische Industrie GmbH, Germany), *EP* 1229127 A1; *Chem. Abstr.* **2002**, *137*, 139499.

[15] H. Spahn-Langguth, *Pharm. Unserer Zeit* **1996**, *25*, 198–219.

[16] W. H. J. Boesten, P. Riebel, G. Niederhumer, (DSM Fine Chemicals Austria Nfg G. m. b. H. + Co. K.-G., Austria), *WO* 2001092199 A1, **2001**; *Chem. Abstr.* **2001**, *136*, 5726.

[17] R. Rothermel, M. Hanack, *Liebigs Ann. Chem.* **1991**, 1013–1020.

[18] A. Wissner, *Tetrahedron Lett.* **1978**, 2749–2752.

[19] S. Abazi, L. Parra Rapado, K. Schenk, P. Renaud, *Eur. J. Org. Chem.* **1999**, 477–483.

[20] W. H. Pearson, M.-C. Cheng, *J. Org. Chem.* **1987**, *52*, 1353–1355.

[21] R. Ramage, A. M. MacLeod, G. W. Rose, *Tetrahedron* **1991**, *47*, 5625–5639.

[22] K. Faber, *Biotransformations in Organic Chemistry*, Springer Verlag, Berlin, Germany, **1997**.

[23] S. M. Roberts, G. Casy, M.-B. Nielsen, *Biocatalysis for Fine Chemicals Synthesis*, John Wiley & Son Ltd, New York, Chichester, Brisbane, Toronto, Singapore, **1999**.

[24] U. T. Bornscheuer, R. J. Kazlauskas, *Hydrolases in Organic Synthesis: Regio- or Stereoselective Biotransformations*, John Wiley & Son Ltd, New York, Chichester, Brisbane, Toronto, Singapore, **1999**.

[25] The following enzymes were used: lipases: *Alcaligines* sp., *Aspergillus niger*, *Aspergillus oryzae*, *Candida antarctica* A, *Candida antarctica* B, *Candida cylindracea*, *Candida lipolytica*, *Candida rugosa*, *Mucor javanicus*, *Mucor miehei*, *Penicillium roqueforti*, *Pseudomonas cepacia*, *Pseudomonas fluorescens*, *Pseudomonas* sp., *Rhizomucor miehei*, *Rhizopus arrhizus*, *Rhizopus niveus*, *Thermomyces lanuginose*, pig pancreas, wheat germ esterases: acetylcholine esterase from *Elektrophorus electricus*, *Bacillus* species, *Bacillus stearothermophilus*, *Bacillus thermoglucosidasius*, *Candida lipolytica*, *Mucor miehei*, *Saccharomyces cerevisiae*, *Thermoanaerobium brockii*, horse liver, pig liver.

[26] C.-S. Chen, Y. Fujimoto, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.

[27] For the calculation of the enantiomeric ratio *E* of a kinetic resolution it is essential to know either the enantiomeric excess (ee) of the substrate and the product or the extent of conversion c together with one enantiomeric excess (substrate or product). In our case, the resolution of **(*R,S*)-2** affords only one chiral "product", the remaining enantiomer. The actual reaction product of the resolution is the aldehyde **6**, which is not chiral. Therefore for the calculation of the *E* value an appropriate method for the determination of the conversion rate was necessary. On an industrial scale the measurement of c with NMR spectroscopic methods is sufficient and, what is more important, easy and fast. On a laboratory scale we used another method. The conversion c was determined using an internal standard. This method provided

reliable and precise data. But due to the fact, that even a small variation of the input values (ee or c) causes a significant change in the numerical value *E*, all stated values were verified by plotting the ee–c diagrams for several extents of conversion so that a best-fit graph could be generated.

[28] E. Holmberg, K. Hult, *Biotechnol. Lett.* **1991**, *13*, 323–326.

[29] A. Liese, K. Seelbach, C. Wandrey, *Industrial Biotransformations*, Wiley-VCH Verlag GmbH, Weinheim, **2000**, p. 61.

[30] The hydroxymethyldioxolanone **4** was identified with NMR spectroscopic methods: ^{1}H NMR: $\delta = 5.70$ (dd, $J_1 = 2.5$ Hz, $J_2 = 2.7$ Hz, 1H, $CHCO_2$), 3.88 (dd, $J_1 = 2.5$ Hz, $J_2 = 12.9$ Hz, 1H, $COO\text{-}CH_2$), 3.83 (dd, $J_1 = 2.7$ Hz, $J_2 = 12.9$ Hz, 1H, $COO\text{-}CH_2$), 4.43 (dd, $J_1 = 14.9$ Hz, $J_2 = 0.9$ Hz, 1H, OCH_2CO), 4.29 (dd, $J_1 = 14.9$ Hz, $J_2 = 0.6$ Hz, 1H, OCH_2CO). A direct detection of **4** with GC or HPLC methods is not possible, but after reaction with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) the silylated compound can be quantitatively determined by GC.

[31] The absolute configuration was derived from the known chirality of **(*R*)-2** under the assumption that the stereospecificity of the lipase does not change under otherwise identical reaction conditions.

