117. Preparation, Structure, and Properties of All Possible Cyclic Dimers (Diolides) of 3-Hydroxybutanoic Acid

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In connection with the proposed structure of a *trans*-membrane cellular ion channel consisting of a complex between poly[(*R*)-3-hydroxy butanoate] (P(3-HB)) and calcium polyphosphate, $CaPP_i$ (*ca.* 150 units each), which is supposed to contain s-*cis*-bonds or even more highly strained ester conformations, we have prepared and studied the properties of the cyclic dimer of 3-HB, the diolide **1**. All possible forms of **1**, the *rac*-, the *meso*-, and the enantiomerically pure (*R,R*)- and (*S,S*)-compounds were prepared, purified, and characterized. The synthesis (*Scheme 1*) started from dimethyl succinate with the key step being the *Baeyer-Villiger* oxidation of the *rac*- and *meso*-2,5-dimethylcyclohexane-1,4-diones **5**. The *rac*-diolide **1** was resolved by preparative chromatography on a *Chiralcel OD* column (*Fig. 1*). The crystal structures of *rac*-**1** (*Fig. 3*) and of *meso*-**1** (*Fig. 5*) were determined by X-ray diffraction: the diolides **1** contain s-*cis*-ester bonds and an ester group with a conformation half way to the transition state of rotation (*Fig. 2*). Strain energies for the diolides **1** of up to 17.8 kcal/mol are suggested. Accordingly, these compounds show reactivities similar to those of carboxylic-acid anhydrides or even acid chlorides. They cannot be chromatographed on silica gel, and they react with primary, secondary, and tertiary alcohols, and with amines to form derivatives of open chain 3-HB 'dimers', hydroxy acids **6**, esters **7**, and amides **8** (*Scheme 2*). The rate of acid-catalyzed ring opening of the diolides **1** with alcohols has been measured (*Figs. 6* and *7*). From the results described, we conclude that it is unlikely for strained and reactive ester conformations to occur as part of ion channels through phospholipid bilayers of cells.

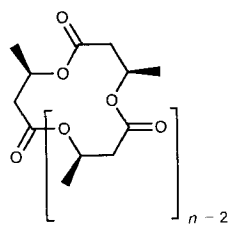
1. Introduction. – In several publications [1–5], we have reported the synthesis, isolation and characterization of macrocyclic oligomers of type **A**, so-called oligolides. They all contain repetitive units of (*R*)-3-hydroxybutanoic acid (3-HB) as the monomeric building block and were obtained by the use of well-established macrolactonization methods. At the beginning of our work [1], we synthesized these oligolides **A** in order to investigate their possible antibiotic activity[2]). Recently, a related group of compounds, the poly(3-hydroxyalkanoates), have become the subject of increasing interest. These polymers are fully biodegradable and cause many expectations in the fields of medicine and materials science [4] [5]. The most important member of this family is poly[(*R*)-3-hydroxybutanoic acid] (P(3-HB)), a material of high molecular weight (1–7.5 × 10^5 g/mol or 2500–9000 monomeric units). A copolymer of P(3-HB) with (*R*)-3-hydroxypentanoic

[1]) Part of the projected dissertations of *T. H.* and *F. N. M. K.*, ETH-Zürich.

[2]) For instance, the tetrolide has a 16-membered ring system which is reminiscent of naturally occurring fungal metabolites such as pyrenophorine, vermiculine, conglobatine, and elaiophylidine.

acid (3-HV)[3]) is produced on a large scale by the fermentation of *Alcaligines eutrophus* by *Zeneca Bio Products* [6]. Besides its application as microbial storage material, P(3-HB) with a comparatively lower molecular weight (*ca.* 10000 g/mol or 100–150 monomeric units) has been found, together with calcium polyphosphate, in extracts from cell membranes of genetically transformable *Escherichia coli* [7–9]. In some extractions performed in our laboratories, we found P(3-HB) containing up to 14% of 3-HV [10]. *Reusch* proposed that this low-molecular-weight P(3-HB) may be part of a non-proteinogenic ion channel, located in the inner cell wall, and be responsible for calcium ion, phosphate, or even DNA transport across the inner bacterial cell wall [11].

During our investigations on the structure and synthesis of this ion channel, we prepared the oligolides **A** containing 3 to 10 monomeric units of 3-HB (12 to 40 ring atoms) and analogous macrocycles with 3 to 12 monomeric units of 3-HV (12 to 48 ring atoms). Because of their defined molecular weight and higher crystallization tendency, we expected these oligolides to be model compounds for the polymeric material. Therefore, we determined several of their X-ray crystal structures which led to the construction of models for the polymer containing a 3_1 and a 2_1 helix. Using the 2_1 helix as a building block, an alternative structure for the channel was proposed [12] [13]. Moreover, we succeeded in crystallizing complexes of the triolides with alkali and alkaline-earth-metal salts, and these have been characterized by X-ray crystal-structure analysis [13] [14]. Finally, some of the oligolides have been used as ionophores to transport ions across a bulk liquid CH_2Cl_2 membrane [15].



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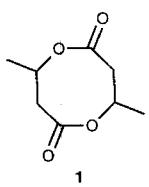

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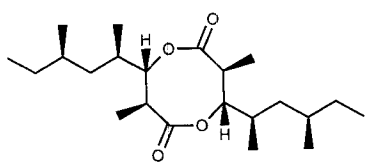

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Usually, mixtures of oligolides **A** were prepared by different macrolactonization methods in a *single* reaction starting from the monomeric (*R*)-3-hydroxybutanoic acid itself. Separation by chromatographic methods afforded pure macrocycles. This means that the open-chain precursor of each oligolide is formed *in situ* by sequential esterification of the hydroxy acid with itself. With all investigated macrolactonization methods used so far, we have been unable to detect the cyclic dimer **1** of 3-HB. There was no spectroscopic evidence for the formation of this compound by either ^{13}C-NMR spectroscopy or LSI-MS[4]) of the crude oligolide mixtures. We expected the diolide **1** to be an

[3]) The abbreviation 3-HV is deduced from the trivial name (*R*)-3-*h*ydroxy*v*aleric acid.

[4]) Liquid secondary ionization mass spectroscopy.

interesting molecule in its own right: to date, no solid-state structure of such a dilactone has been described. Only the calculated (MMX 87 force field) minimum-energy conformation of a related ring system (the diolide **2** of bourgeanic acid) has been published [16]. Moreover, the skeleton of this diolide molecule should be strained due to unfavorable ester conformations imposed by the eight-membered-ring structure (see *Sect. 3*). Therefore, the attack of a nucleophile at the carbonyl-C-atom of such a cyclic dimer will be favored which gives an additional feature to the molecule: by a ring-opening reaction, a dimeric unit of (*R*)-3-hydroxybutanoic acid can be coupled to that nucleophile in just one step.

2. Synthesis and Separation of the Three Possible Stereoisomeric Diolides. – When we began with the synthesis of the diolide **1**, we followed the most convenient strategy: namely, to synthesize the open-chain dimeric ω-hydroxy acid and to cyclize it. Two lactonization procedures, which turned out to be very useful for the preparation of oligolides **A** starting from the monomeric hydroxy acid, have been studied in detail for this purpose: *a*) *Yamaguchi*'s method *via* the mixed carboxylic 2,6-dichlorobenzoic anhydride in the presence of a 4-(dialkylamino)pyridine under high-dilution conditions [17], and *b*) the *N,N'*-dicyclohexylcarbodiimide(DCC)-induced lactonization with catalytic amounts of a 4-(dialkylamino)pyridine [18]. However, the analyses of product mixtures resulting from the reactions of the dimeric ω-hydroxy acid were rather disappointing. Using *Yamaguchi*'s method, *ca.* 20% of crotonic acid was isolated, accompanied by the cyclic oligomers **A** with 4, 5, 6, 7, and 8 monomeric units. Presumably, the activated open-chain 'dimer' of 3-HB decomposes by β-elimination and the (*R*)-3-hydroxybutanoic acid formed *in situ* can be activated again which finally leads also to oligolides with an odd number of monomeric units. The attempts with DCC-activated lactonizations gave nearly the same product ratios as described above. Variation of the reaction conditions (temperature, solvent, catalyst, concentration of the activating reagent, or of the open-chain precursor) did not lead to detectable amounts of the diolide **1**.

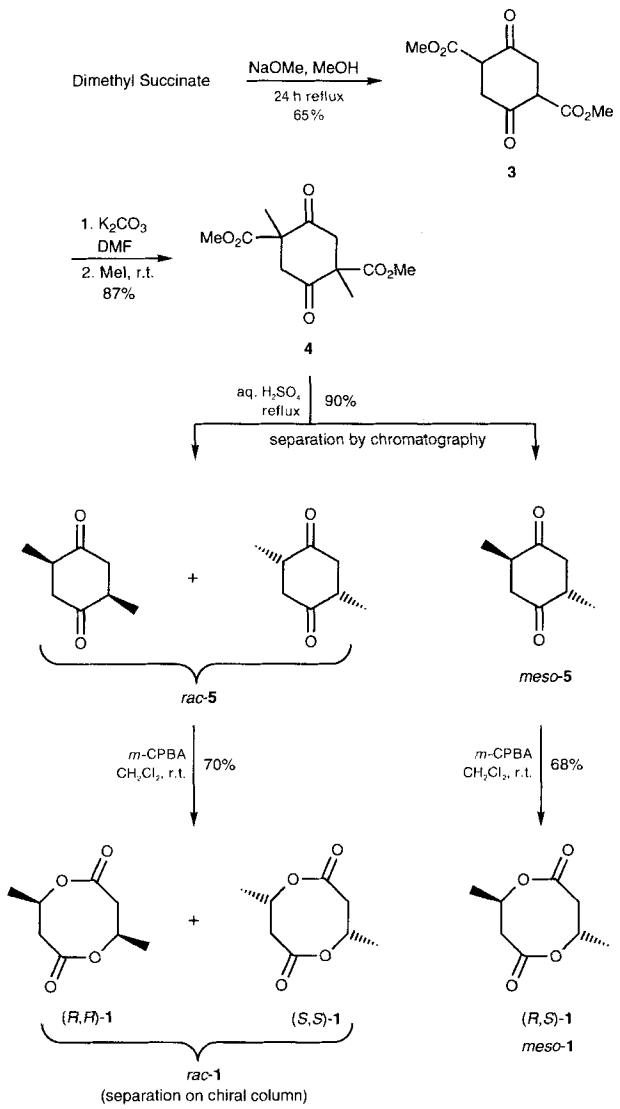
Only a few publications deal with the synthesis of such dilactones derived from 3-hydroxy acids [16] [19–24]. We suspect that, in some of these investigations, the diolides might have been mistaken for higher oligolides. The most striking one describes the discovery of the dilactone **2** as a minor component during the isolation of the lichen metabolite of (+)-bourgeanic acid [22] [23]. More recently, *White* and *Johnson* improved the synthesis of this eight-membered dilactone by applying milder conditions [16] [24]. In our subsequent experiments, these same reaction conditions were applied in the attempted synthesis of **1**. All methods failed. Interestingly, the procedure described by *White* and *Johnson* gave only the triolide of HB in *ca.* 50% yield, and this was the only method to yield a cyclic product. However, it should be noted that the dilactone derived from bourgeanic acid contains additional Me groups in the α-position, whereas the diolide **1** is unsubstituted there. This substitution might be expected to lead to a manifestation of the 'reactive rotamer effect' [25].

On the basis of these observations, we changed our initial strategy and decided to build up this eight-membered cyclic system by a ring-enlargement reaction [26]. In many publications, it has been demonstrated that the classical *Baeyer-Villiger* oxidation offers the best synthetic method for lactone formation of medium ring size [27]. A number of oxidation reagents are known, and these have been summarized in a recent excellent review [28]. Since this method had been used previously for the preparation of eight-mem-

bered catechol dilactones [29], we expected it to be the most efficient method for the synthesis of *rac*-**1** and *meso*-**1**.

As shown in *Scheme 1*, the cyclic dione **5** was easily prepared from dimethyl succinate in an overall yield of 51%. The first step consisted of a *Claisen* condensation, followed by a *Dieckmann* cyclization to give **3** [30]. After recrystallization, **3** was regioselectively dimethylated with MeI in DMF under basic reaction conditions to give **4** as a 7:3 mixture of diastereoisomers. Acid-catalyzed hydrolysis of the methyl-ester groups and decar-

Scheme 1. *Synthesis of* rac-**1** *and* meso-**1** *Starting from Dimethyl Succinate*. The key reaction step is a *Baeyer-Villiger* oxidation. *m*-CPBA = *m*-chloroperoxybenzoic acid.



boxylation [31] of the two resulting β-keto-acid moieties led to a mixture of *rac*-**5** and *meso*-**5**, which had been previously isolated and their configurations assigned by *Stolow* and *Bonaventura* [32]. Interestingly, the *cis*-dione *rac*-**5** predominates (80%), a finding that has been rationalized [32] with a twist-boat-type conformation of the six-membered dione ring [33]. The mixture of *rac*-**5** and *meso*-**5** was separated by careful flash chromatography. Treatment of *rac*-**5** or *meso*-**5** with 3 equiv. of *m*-chloroperoxybenzoic acid (*m*-CPBA) at room temperature for 2 d in the dark gave the corresponding dilactones *rac*-**1** or *meso*-**1** in *ca.* 70% yield of purified material. It should be noted at this point that the rearrangement occurred with complete regio- and stereoselectivity, following the rules of sextet rearrangements[5]): *rac*-**5** gave *rac*-**1**, and *meso*-**5** gave *meso*-**1** exclusively, and this was proven as follows.

It was found that the most effective method of enantiomer separation of *rac*-**1** was by chromatography using the chiral stationary phase *Chiralcel OD*, a carbamate derivative coated on silica gel. Attempts to separate the enantiomers of *rac*-**5** on a preparative scale, either by enantioselective protonation of the dienolate or by crystallization of diastereo-

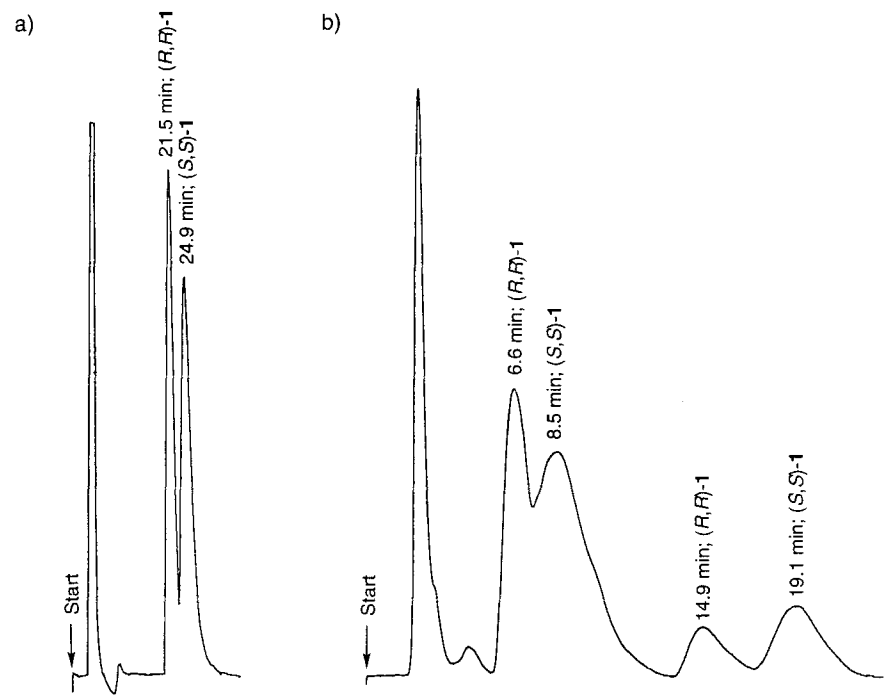


Fig. 1. *Resolution of* rac-**1** *by HPLC on* Chiralcel OD. *a*) Analytical: 250 × 4.6 mm; 10-μm particle size; eluent hexane/i-PrOH 99.4:0.6; flow 1 ml/min; detection at 216 nm. *b*) Preparative: 250 × 50 mm; 20-μm particle size; eluent heptane/i-PrOH 99:1; flow 150 ml/min; detection at 216 nm. Injection of 200 mg of *rac*-**1** dissolved in 1 ml of CH_2Cl_2. The peak shaving was performed by manual separation of the fronts and tails and led to enantiomerically pure (*R,R*)-**1** (first peak) and (*S,S*)-**1** (assignment of chirality sense, see accompanying paragraph).

[5]) Examples of enantioselective *Baeyer-Villiger* oxidations are known [34], but the selectivities are only high in those reactions employing enzymes [35].

isomeric derivatives (for example, the diacetals formed with tartaric acid) failed. For the chromatographic enantiomer separation of *rac*-**1**, we used the closed-loop recycling mode [36–38] (see Fig. 1 in [39]). This special kind of HPLC technique became necessary because of the rather small separation factor $\alpha = 1.2$. After injection of the sample, the elution was followed by UV spectroscopy. The front of the first peak was collected, while the following mixed fractions were recycled back onto the column. The tail of the second peak was also collected. In the following cycles, this process was repeated as often as necessary.

In *Fig. 1*, an analytical HPLC run is compared to the corresponding preparative one. *Chiralcel OD* of 10-μm (analytical) and of 20-μm (preparative) particle size was used. The advantage of this HPLC technique becomes clearly evident: by peak shaving, the enantiomers of *rac*-**1** appear as base-line-separated peaks after the second cycle. The absolute configuration of the separated enantiomers was assigned by hydrolysis of the laevorotatory, first eluted diolide (*R,R*)-**1** to the known [40] open-chain 'dimeric' hydroxy acid (*R,R*)-**6** (general formula, see *Scheme 2*, below). From this assignment, the configurations of the other two forms of **1** have been established, as well as those of the precursors *rac*-**5** and *meso*-**5**.

3. Crystal Structures of *rac*- and *meso*-Diolide 1: Two Sorts of Highly Strained Ester Bonds. – The only structure proposal for an eight-membered dilactone similar to **1** given in the literature is for the previously discussed cyclization product **2** of bourgeanic acid [16]. As already mentioned, unlike our dilactone **1**, this cyclic diester is substituted on both C-atoms α to the C=O groups, and these substituents may play a considerable role in determining the conformation adopted by the dilactone ring. Furthermore, the structure was not determined but calculated (MMX 87 force field); the resulting minimum-energy conformation is a crown-type arrangement, which is usually unfavorable for eight-membered rings containing zero, one, or two heteroatoms [33]. Therefore, this proposed structure seemed to be a rather unsuitable model for the structure of *rac*-**1** and *meso*-**1**. Moreover, we were interested in determining the actual conformation of the ester bonds, because *Reusch*'s proposed model of an ion channel [11], which has been alluded to in *Sect. 1*, is constructed from 3-HB units which contain s-*cis*-ester bonds and even ester bonds at the transition state of rotation between *cis* and *trans* (see *Fig. 2*). As both *rac*-**1** and *meso*-**1** were isolated as colorless crystalline materials, suitable crystals for X-ray crystal-structure analysis were obtained by careful recrystallization from Et_2O/pentane mixtures.

The almost perfectly C_2-symmetric structure of the *rac*-diolide **1** can be described as an eight-membered ring built from two planar halves which are two s-*cis*-ester groups. They are connected head to tail by two C–C bonds and are tilted away from each other in

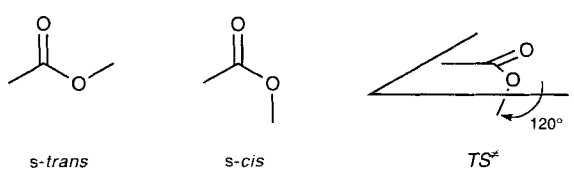


Fig. 2. *Two stable conformations of ester bonds and the transition state of rotation.* The relative energies for methyl acetate are: *trans*, 0; *cis*, 8.5; TS ≠, 13.4 kcal/mol [47].

such a way that, in analogy to cyclohexane, the conformation may be designated as boat form (*Fig. 3*).

The ester groups are slightly *staggered* to avoid the *Pitzer* strain between the CH protons and the axial CH_2 protons. The *Newman* projection along the sp^3-sp^3 C–C bond connecting the ester groups shows an arrangement falling somewhere between *staggered* and *eclipsed* (H–C–C–H dihedral angles 33.9 and 38.3°). This puts both Me groups in an equatorial position. Consequently, the CH protons are axial and point under and inside the ring. The distance between these protons is 2.09 Å which is less than the sum of their *van der Waals* radii, so there must be considerable transannular strain in the molecule. As far as the bond lengths and angles are concerned, the observed values fit very well with the average values found by *Dunitz* and *Schweizer* when they analyzed the data of over a thousand ester structures retrieved from the *Cambridge Structural Database* (*CSD*) [41]. Only the C–C–C bond angles inside the ring are wider than the expected 109°, but the deviation to 117.8 and 119.3° cannot be considered severe enough to drastically increase the molecule's strain energy. The energetically most unfavorable parts of the structure are the s-*cis*-ester groups, which deviate slightly from planarity (torsion angles $\tau = 172$ and 175°). This ester conformation has been found only in four- to seven-membered lactones [42]. Already, in the case of the eight-membered lactone ring, both conformations – s-*cis* [43] and s-*trans* [44] – have been found in crystal structures. For the ten-membered ring of nonanolactone, only the s-*trans*-conformation is observed in the X-ray structure [45]. In the database search by *Schweizer* and *Dunitz* [41], not a single example of an acyclic s-*cis*-ester appears. These observations may easily be explained by the unfavorably high energy of the s-*cis*-ester conformation. For example, in the case of methyl acetate [46], the measured value was found to be 8.5 kcal/mol higher than the value for the s-*trans*-form, and this value has been reproduced by 6-311G* calculations [47]. Bearing in mind that both ester groups of the molecule exist in the s-*cis*-conformation, the strain energy must be higher than 17 kcal/mol when compared to an open-chain 3-HB 'dimer'. The only structure comparable to this small ring dilactone is the one of the corresponding dilactam, cyclo(di-β-alanyl) [48]. A superposition of both molecules is shown in *Fig. 4, c*. The best fit of both structures shows excellent agreement, but the amide, with a melting point of 299°, can be recrystallized from boiling water [49], whereas **1** readily decomposes in refluxing hexanes.

In the structure of the *meso*-diolide **1**, the situation is even more unusual. In fact, the molecule has a typical boat-chair conformation (*Figs. 4* and *5*), which is the lowest-energy conformation for an eight-membered ring [33].

A search in the *CSD* gave three structures of cyclooctane-1,5-diones [50–52], a superposition of which with *meso*-**1** is depicted in *Fig. 4, d*. Neither a corresponding oxo-lactone nor a dilactone structure containing eight ring atoms was found in the *CSD*. However, both C=O groups occupy the positions which are subject to the strongest transannular interactions within cyclooctane, and while one ester bond is s-*cis*, the other, which has a torsion angle of 49°, adopts a conformation almost half way to that of the transition state of the ester-bond rotation (120°, see *Fig. 2*). A conformation with a torsion angle of 60° was calculated by *Wiberg* and *Laidig* [47] to be 9.34 kcal/mol higher in energy than the normal s-*trans*-arrangement. Adding the 8.5 kcal/mol for the s-*cis*-ester bond, the total strain energy is estimated to be 17.8 kcal/mol. However, this high level of conformational strain energy seems to be compensated by other structural features.

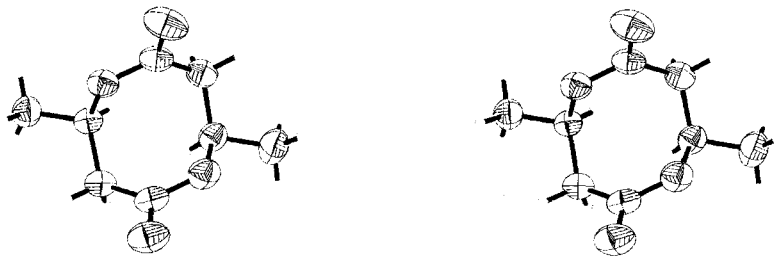


Fig. 3. *Stereoscopic ORTEP plot of* rac-**1**. The O-atoms are shown in red, C-atoms in black, and H-atoms in green. The thermal elipsoides are drawn to the 50% probability level.

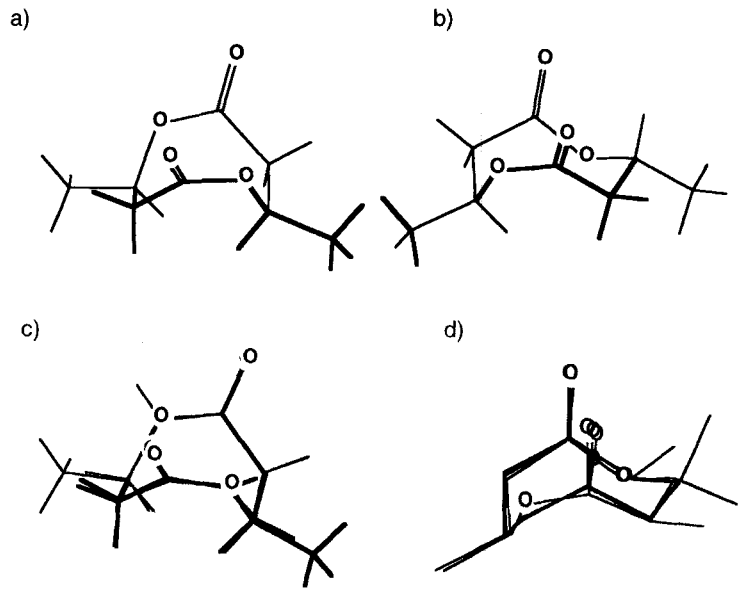


Fig. 4. *Crystal structures shown as MacMoMo representations. a) rac-***1** in its boat-type conformation. *b) meso-***1** in its boat-chair conformation. *c*) Superposition of *rac-***1** (red) and cyclo(di-β-alanyl) (black). *d*) Superposition of *meso-***1** (red), cyclooctane-1,5-dione [50], 3,7-dimethylcyclooctane-1,5-dione [51], and 2,4-dimethylcyclooctane-1,5-dione [52].

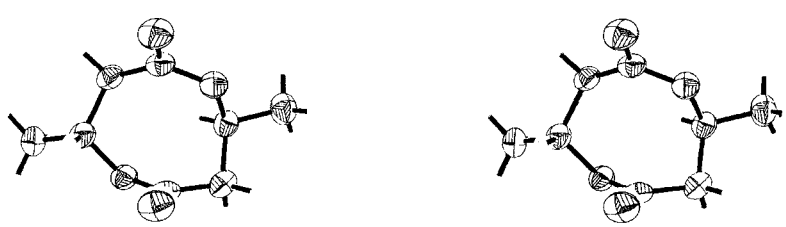


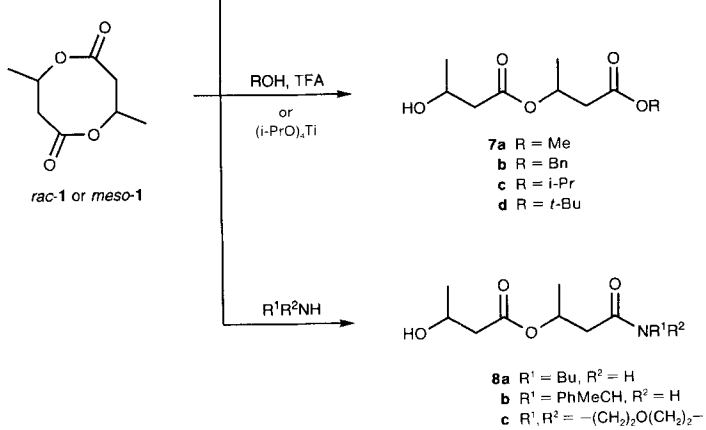
Fig. 5. *Stereoscopic ORTEP plot of* meso-**1**. The O-atoms are shown in red, C-atoms in black, and H-atoms in green. The thermal elipsoides are drawn to the 50% probability level.

These include the Me substituents in equatorial positions on the eight-membered ring, the sp^3-sp^3 C–C bonds being staggered (H–C–C–H dihedral angles 57.4 and 68.3°), and also the bond lengths and angles are in good agreement with the average values for carboxylates [41]. Only the bond angle at one of the O-atoms in the ring is slightly wider (124°) than that found in the unstrained situation (117°) [41]. The two axial H-atoms on both sides of the s-*cis*-ester bond are pointing underneath and inside the ring (*Fig. 4, b*) but do not really come close enough for a strong transannular interaction (2.12 Å).

The diolides *rac*-**1** and *meso*-**1** differ only by one stereocenter, but the X-ray structures of the molecules show completely different conformations. The most striking common structural feature is the equatorial position of the Me substituents. An axial Me group on these eight-membered rings would cause such unbearable strain that even s-*cis*-ester bond torsion angles are tolerated to avoid it.

4. Some Reactions of the Diolides 1 with Nucleophiles. – As pointed out in the previous section, *rac*-**1** and *meso*-**1** have unfavorable ester conformations, and, therefore, it is not surprising that these molecules react rapidly with nucleophiles [42a] as shown in *Scheme 2*. It has proved impossible to isolate the purified compounds by chromatography on silica gel – presumably, a ring opening reaction takes place with traces of H_2O under the slightly acidic conditions, or else, the silica gel OH groups are esterified to give $Si{-}OCOCH_2CH(Me)O{-}COCH_2CH(Me)OH$. However *rac*-**1** and *meso*-**1** are stable enough to be recrystallized from solvents with low boiling points, such as pentane or Et_2O, while slow decomposition is observed in boiling hexanes.

Scheme 2. *Some Ring-Opening Reactions of* rac-**1** *and* meso-**1** *with Nucleophiles.* In the case of *rac*-**1**, the resulting open-chain dimeric hydroxy acid-ester and -amide derivatives consist of homochiral and, in the case of *meso*-**1**, of heterochiral building blocks. See also *Figs. 6* and *7*.



Initially, the reactivity of *rac*-**1** and *meso*-**1** with oxygen nucleophiles was investigated. Stirring with H_2O in the presence of silica gel at room temperature gives the open chain 'dimeric' hydroxy acid **6** of 3-HB within 3 h. This ring opening can also be performed by dissolving *rac*-**1** in aqueous THF (*ca.* 1% of H_2O) and adding catalytic amounts of AcOH or CF_3COOH (TFA). The absolute configuration of (*S*,*S*)-**1** was assigned by this hydrolysis procedure (see *Sect. 2*). A variety of alcohols are capable of attacking the C=O C-atoms of these diolides. Primary alcohols, such as MeOH or BnOH, react completely with *rac*-**1** and *meso*-**1** to produce the corresponding ω-hydroxy esters **7a** and **7b**. ^{1}H-NMR Spectroscopy (*Fig. 6, a*) was used to investigate the kinetics of the methanolysis reaction of *rac*-**1**. The acid-catalyzed (TFA) reaction of *rac*-**1** with 7 equiv. of MeOH in $CDCl_3$ at room temperature was monitored. As can be seen, there was 50% conversion after 4 min, 60% after 6 min, and 90% after 15 min reaction time. A comparison with the analogous reaction of MeOH in $CDCl_3$ with *meso*-**1** showed a slightly reduced reaction rate. In this case, 50% conversion was achieved after 8 min and 78% after 15 min – this is an unexpected result as the solid-state structure of *meso*-**1** indicates a slightly more strained conformation of the eight-membered ring. On the other hand, the NMR spectra[6]) of both diastereoisomers *rac*-**1** and *meso*-**1** show that these molecules have an average C_2 and C_i symmetry on the NMR time scale. In *Fig. 6, b*, the course of the methanolysis of *rac*-**1** is compared to that of *meso*-**1** as a function of time.

Similar ^{1}H-NMR investigations were carried out using only 1 equiv. of the alcohol. This allowed a comparison of the reactivity of primary, secondary, and tertiary alcohols. As shown in *Fig. 7*, primary alcohols were found to have the highest reactivity (with MeOH, 50% of *rac*-**1** remained after 1 h), secondary alcohols were considerably less reactive (with i-PrOH, 92% of *rac*-**1** remained after 1 h), and tertiary alcohols showed the lowest reactivity (with *t*-BuOH, 98% of *rac*-**1** remained after 1 h). By assuming second-order kinetics, the rate constants *k* for the reactions of the three alcohols were calculated and, as can be seen from the table in *Fig. 7*, the *r* factors confirm this as a reasonable assumption. The considerably higher *k* value found for MeOH clearly demonstrates the greater reactivity of the primary alcohol, which suggests that it may be possible to selectively couple a primary alcohol to a 3-hydroxybutanoic acid 'dimer' in the presence of a secondary- or tertiary-alcohol function. This was indeed found to be the case in the acid-catalyzed reaction of 2 equiv. of an equimolar mixture of MeOH and *t*-BuOH with *rac*-**1** which, after 5 h, yielded 78% of **7a** and only 4% of **7d**.

We have also studied the ring-opening reaction with $(i\text{-PrO})_4Ti$ [53]: the addition of 1 equiv. of $(i\text{-PrO})_4Ti$ at room temperature led to the formation of 50% of **7c** after 6 min and 85% after 20 min. The corresponding experiment with *meso*-**1** gave a similar result. Thus, the reaction mediated by an equivalent amount of *Lewis* acid is much faster than the proton-acid-catalyzed one.

In principle, amines are capable of cleaving carboxylic-ester bonds to give the corresponding amides. Although amide-bond formation is thermodynamically favored, aminolysis reactions with non-activated esters, such as methyl or ethyl esters, are often very slow. Activated esters and anhydrides, however, react completely to give the desired

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[6]) The spectrum of *meso*-**1** was measured at temperatures as low as −90° (CD_2Cl_2): there was some line broadening but no splitting of signals which would indicate the presence of a slowly equilibrating C_1 symmetrical species.

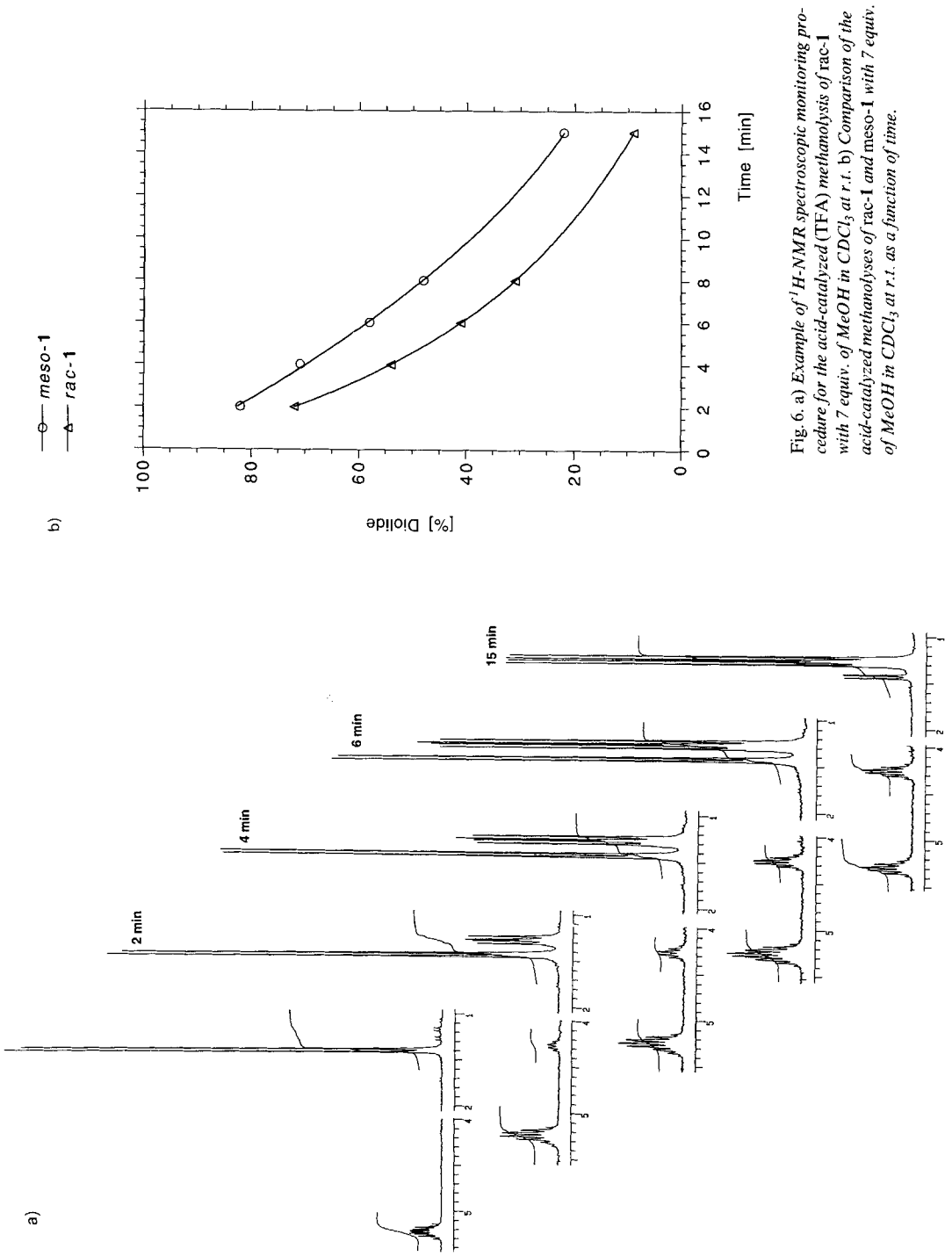
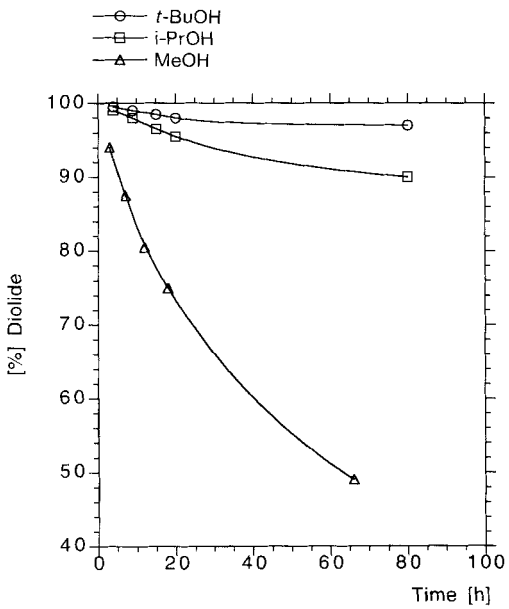


Fig. 6. a) *Example of ^{1}H-NMR spectroscopic monitoring procedure for the acid-catalyzed* (TFA) *methanolysis of* rac-**1** *with 7 equiv. of MeOH in $CDCl_3$ at r.t.* b) *Comparison of the acid-catalyzed methanolyses of* rac-**1** *and* meso-**1** *with 7 equiv. of MeOH in $CDCl_3$ at r.t. as a function of time.*



Entry	Alcohol	*k* Value [l·mol^{-1}·min^{-1}]	*r* Factor
1	MeOH	0.225	0.998
2	i-PrOH	0.027	0.995
3	*t*-BuOH	0.012	0.998

Fig. 7. *Acid-catalyzed (TFA) alcoholysis reactions of* rac-**1** *with 1 equiv. of MeOH, i-PrOH, or* t-*BuOH at r.t. as a function of time. k* Values and *r* factors for these reactions. Assuming second-order kinetics, the values were obtained by linearization: $1/c - 1/c_0 = kt$.

amides, and this led us also to study the reactivity of *rac*-**1** with nitrogen nucleophiles. In the presence of 4 equiv. of $BuNH_2$, 25% of the amide **8a** was found after 4 h at room temperature, and this increased to 70% after 24 h with no products from side reactions, such as ring opening by β-elimination, being observed. α-Branched primary amines, which are more sterically hindered, are able to attack the C=O C-atom of *rac*-**1** as well: 1-phenethylamine (1 equiv.) gave 50% of **8b** after 10 d at room temperature. This reaction was analyzed to see whether the possible kinetic resolution of the enantiomers of *rac*-**1** with (*R*)-phenethylamine occurs, but no diastereoselectivity was found. Finally, **1** can also be converted to amides of secondary amines. The reaction of *rac*-**1** with 4 equiv. of morpholine gave 50% of **8c** after 2 d, while after 6 d, 94% of the amide **8c** had been formed[7]).

5. Conclusions. – This work has demonstrated that the synthesis of the cyclic dimers of 3-hydroxybutanoic acid, the diolides **1**, cannot be achieved by lactonization of the open-chain precursor using the methods investigated. Obviously, such a linear 'dimer' **6** does not adopt the highly strained conformation which is necessary for the ring-closure step. It seems, instead, to prefer the s-*trans*-conformation for the ester group. The surprising finding of *White* and *Johnson* [16] [24], the ease with which the corresponding dilactone **2** of bourgeanic acid is formed, must be due to the effect of the substituents [25] [54]. A similar observation has been reported by *Adam et al.* in their synthesis of

[7]) As a side product, 3% of 3-(crotonyloxy)butyric acid, the product of eliminative ring opening, was detected.

β-lactones from β-hydroxy acids under otherwise identical conditions: if the α-substituent was missing, no cyclic product was formed [55]. However, we could obtain the strained eight-membered ring skeleton of the diolides **1** in high yields by a ring-enlargement reaction. The described ring openings by nucleophiles showed that the reactivity [42a] of *rac*-**1** and *meso*-**1** falls somewhere between that of an acid anhydride and an acid chloride.

The high reactivities of *rac*-**1** and *meso*-**1** are reflected in their X-ray crystal structures which show highly unfavorable ester conformations. These results are in contrast to *Reusch*'s proposed channel model containing s-*cis*-esters and even ester conformations close to the transition state of bond rotation [11]. We believe that the presumed function of oligo(3-HB) in cell walls as part of a non-proteinogenic ion channel can only be realized, if the oligomer chain adopts a backbone containing ester groups of s-*trans*-conformation. An alternative channel model, which is in accord with this requirement has been suggested by us [12] [13].

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Experimental Part

1. *General.* All solvents were either *puriss p.a.* quality or distilled over appropriate drying agents. TLC: *Merck-TLC-F*$_{254}$ precoated glass plates; detection by UV_{254} light, immersing in an iodine bath (30 g I_2, 2 g KI, in 400 ml EtOH/H_2O 1:1) and warming or staining with phosphomolybdic acid (25 g of phosphomolybdic acid, 10 g of $Ce(SO_4)_2 \cdot 4\,H_2O$, 60 ml of conc. H_2SO_4, and 940 ml of H_2O). Flash chromatography (FC): silica gel *60* (*Merck*) 40–63 μm. Anal. high-pressure liquid chromatography (HPLC) was performed by employing a *Kontron* HPLC system (UV detector *Uvikon LCD-75*, *Programmer 200*, integrator *Shimadzu C-R 1B Chromatopak*) using a *Daicel* column (*Chiralcel OD*, 250 × 4.6 mm, 10 μm). M.p.: *Büchi 510*; not corrected. $[\alpha]_D$: *Perkin-Elmer 241* polarimeter. IR Spectroscopy: *Perkin-Elmer 297* spectrometer; in KBr or $CHCl_3$ soln. ^{1}H- and ^{13}C-NMR spectroscopy: *Bruker WH-300*. All spectra were recorded using $CDCl_3$ as solvent and TMS as internal standard; δ in ppm relative to TMS and J in Hz. Mass spectra: liquid secondary ionization (LSI-MS): *VG-ZAB2-SEQ* with 3-nitrobenzyl alcohol. Elemental analysis: Microanalytical Laboratory of the ETH-Zürich.

2. *Dimethyl 2,5-Dioxocyclohexane-1,4-dicarboxylate* (**3**). A soln. of NaOMe was prepared by adding 32.2 g (1.4 mol) of Na pieces to 230 ml of abs. MeOH at r.t. The reaction was completed by heating the mixture under reflux for 4 h. To the hot soln., 102.3 g (0.7 mol) of dimethyl succinate was added in one portion (exothermic!) and heated at the original bath temp. for additional 20 h. The workup was similar to that given in [30] for the dicarboethoxy derivative and led to 50.3–52.5 g (63.0–65.7%) of **3**. M.p. 155.0–155.5° ([56]: 155.5–157°).

3. *Dimethyl 1,4-Dimethyl-2,5-dioxocyclohexane-1,4-dicarboxylate* (**4**). To a stirred suspension of 62.1 g (0.45 mol) of K_2CO_3 in 1 l of DMF under Ar were added 34.2 g (0.15 mol) of **3**. After 15 min stirring at r.t., 85.2 g (0.60 mol) of MeI were added dropwise. After 15 h, the mixture was concentrated *in vacuo*, dissolved in 300 ml of H_2O, and extracted five times with 500 ml of CH_2Cl_2. The combined org. layers were washed with 10% $Na_2S_2O_3$ soln., dried ($MgSO_4$), and evaporated. The residue was filtered chromatographically (*ca.* 150 g of SiO_2; Et_2O/pentane 4:1) and yielded 33.2–33.5 g (86.4–87.2%) of **4** as a 7:3 mixture of diastereoisomers. M.p. 78.0–79.5°. IR ($CHCl_3$) of the mixture: 3008*w*, 2956*w*, 1745*vs*, 1723*vs*, 1458*m*, 1436*m*, 1113*m*, 986*w*, 849*w*. ^{1}H-NMR (300 MHz), main diastereoisomer: 3.75 (*s*, MeO); $\nu_A = 3.45$, $\nu_B = 2.61$ (*AB*, $J_{AB} = 15.64$, CH_2); 1.42 (*s*, Me). ^{1}H-NMR (300 MHz), minor diastereoisomer: 3.73 (*s*, MeO); $\nu_A = 3.16$, $\nu_B = 2.82$ (*AB*, $J_{AB} = 15.12$, CH_2); 1.45 (*s*, Me). ^{13}C-NMR (75 MHz), main diastereoisomer: 202.56; 170.80; 56.44; 53.27; 47.13; 21.61. ^{13}C-NMR (75 MHz), minor diastereoisomer: 201.86; 171.37; 57.40; 53.27; 47.98; 20.76. EI-MS, mixture: 256.1 (11, M^+), 228.1 (14), 224.1 (34), 197.1 (70), 196.1 (58), 165.1 (17), 164.1 (98), 155.1 (38), 150.0 (17), 137.1 (63), 136.1 (15), 128.1 (52), 127.1 (17), 113.0 (14), 101.1 (17), 100.1 (57), 99.1 (12), 69.0 (100), 59.0 (14), 41.0 (43), 39.0 (14). Anal. calc. for $C_{12}H_{16}O_6$, mixture: C 56.25, H 6.29; found: C 56.20, H 6.28.

4. *2,5-Dimethylcyclohexane-1,4-dione* (**5**). To a stirred suspension of 20.5 g (80 mmol) of **4** in 280 ml of conc. H_2SO_4 were added 5 ml of MeOH and 300 g of crushed ice. After 15 min, this mixture was heated to 100° for additional 2 h. The acidic soln. was cooled to r.t., neutralized with NaOH (pH 6–7), filtered, and extracted three times with 300 ml of CH_2Cl_2. The $Na_2SO_4 \cdot 10\ H_2O$ was stirred with 500 ml of CH_2Cl_2 and filtered. The combined org. layers were dried ($MgSO_4$) and evaporated to yield 10.9–11.0 g (97.1–98.1%) of *rac*-**5** (80%) and *meso*-**5** (20%). FC (1200 g of SiO_2; Et_2O/pentane 1:3) led to 7.8–7.9 g (70.0–70.4%) of *rac*-**5**, m.p. 88.5–89.0° ([32]: 85.0–87.0°) and 1.9–2.0 g (16.9–17.8%) of *meso*-**5**, m.p. 119.0–120.0° ([32]: 119.5–120°).

5. *4,8-Dimethyldioxocane-2,6-dione* (**1**). To a soln. of 1.40 g (10 mmol) of *rac*-**5** or *meso*-**5** in 50 ml of CH_2Cl_2 under Ar were added 7.40 g (30 mmol) of technical *m*-CPBA (70%) in one portion. The pale yellow soln. was stirred at r.t. in the dark for 48 h. After this period, the obtained white suspension was diluted with 50 ml of CH_2Cl_2, washed three times with 100 ml of sat. $NaHCO_3$ soln., which contained 5% of $Na_2S_2O_3$, dried ($MgSO_4$), and evaporated. Recrystallization of the residue from Et_2O/pentane 1:2 gave 1.20–1.23 g (69.7–71.4%) of *rac*-**1** or 1.16–1.18 g (67.4–68.5%) of *meso*-**1**, resp.

6. *Anal. Data. rac*-**1**: M.p. 125.0–125.5°. IR ($CHCl_3$): 3010*w*, 2989*w*, 1750*vs*, 1385*m*, 1283*m*, 1165*s*, 1101*m*, 965*m*. ^{1}H-NMR (300 MHz): 5.34–5.24 (*m*, MeC*H*O). $\nu_A = 2.66$, $\nu_B = 2.55$ (*AB* of *ABX*, J_{ABX}, $J_{AB} = 11.49$, $J_{AX} = 9.72$, $J_{BX} = 3.55$, CH_2); 1.45 (*d*, $J = 6.36$, Me). ^{13}C-NMR (75 MHz): 173.99; 74.72; 44.74; 21.04. LSI-MS: 173.06 (23, $[M + H]^+$), 154.03 (100, $[M - H_2O]^+$). Anal. calc. for $C_8H_{12}O_4$: C 55.81, H 7.02; found: C 55.95, H 6.86.

meso-**1**: M.p. 77.5–78°. IR ($CHCl_3$): 3010*w*, 2987*w*, 1746*vs*, 1385*m*, 1274*m*, 1162*s*, 1120*m*, 963*m*. ^{1}H-NMR (300 MHz): 5.31–5.21 (*m*, MeC*H*O); $\nu_A = 2.91$, $\nu_B = 2.58$ (*AB* of *ABX*, J_{ABX}, $J_{AB} = 13.01$, $J_{AX} = 5.03$, $J_{BX} = 7.98$, CH_2); 1.45 (*d*, $J = 6.42$, Me). ^{13}C-NMR (75 MHz): 172.52; 73.75; 43.86; 21.21. LSI-MS: 173.07 (73, $[M + H]^+$), 154.04 (97, $[M - H_2O]^+$). Anal. calc. for $C_8H_{12}O_4$: C 55.81, H 7.02; found: C 55.91, H 7.10.

7. *Recycling Chromatography*. The following system was used for the separation of the enantiomers with the closed loop technique: *Shimadzu LC-8A* pump, *Knauer* Variable Wavelength Monitor (detection at 216 nm), *Merck Hitachi D-2500* Chromato-Integrator. The separation was performed by employing a *Daicel* column (*Chiralcel OD*, 250 × 50 mm, 20 μm), and injection of 200 mg of *rac*-**1** dissolved in 1 ml of CH_2Cl_2. After one recycling run and manual peak shaving of the head and the tail of each peak, 20 mg (10%) of (*R,R*)-**1** and 18 mg (9%) of (*S,S*)-**1** were isolated in pure form (er > 99:1). (*R,R*)-**1**: M.p. 124.5–125.5° $[\alpha]_D = -91.7$ ($c = 1.145$, $CHCl_3$).

8. *Crystal Structure Analyses*. 8.1. *rac*-**1** ($C_8H_{12}O_4$). The determination of the cell parameters and the collection of the reflection intensities were performed on an *Enraf-Nonius-CAD4* four-cycle diffractometer (graphite monochromatized MoK_α radiation, $\lambda = 0.7107$ Å). Monoclinic, space group $P2_1/n$, $a = 9.219(2)$ Å, $b = 6.466(2)$ Å, $c = 14.973(2)$ Å, $\beta = 104.24(2)°$, $V = 865.1(3)$ Å^3, $Z = 4$, $\rho_{calc.} = 1.322$ gcm^{-3}, $\mu = 0.106$ mm^{-1}, $F(000) = 368$, number of reflections measured 1684 (ω scan, $2 < 2\theta < 52°$), 1684 unique reflections of which 1237 with $I > 3\sigma(I)$ were used for the structure determination (direct methods, SHELXS-86). SHELXL-93 was used for the structure refinement, the non-H-atoms were refined anisotropically. The H-atoms were located from differential *Fourier* syntheses and refined isotropically. Neither absorption nor extinction correction was applied. The refinement converged to $R = 0.0336$ ($wR^2 = 0.0830$, number of variables 157, rest electron density 0.126 and −0.155 e/Å^3).

8.2. *meso*-**1** ($C_8H_{12}O_4$). The determination of the cell parameters and the collection of the reflection intensities were performed on an *Enraf-Nonius-CAD4* four-cycle diffractometer (graphite monochromatized CuK_α radiation, $\lambda = 1.5418$ Å). Monoclinic, space group $P2_1/c$, $a = 9.093(2)$ Å, $b = 9.6924(11)$ Å, $c = 10.080(5)$ Å, $\beta = 100.81(3)°$, $V = 872.5(5)$ Å^3, $Z = 4$, $\rho_{calc.} = 1.311$ gcm^{-3}, $\mu = 0.891$ mm^{-1}, $F(000) = 368$, number of reflections measured 1274 ($\omega/2\theta$ scan, $2 < 2\theta < 120°$), 1274 unique reflections of which 1181 with $I > 3\sigma(I)$ were used for the structure determination (direct methods, SHELXS-86). SHELXL-93 was used for the structure refinement, the non-H-atoms were refined anisotropically. The H-atoms were located from differential *Fourier* syntheses and refined isotropically. No absorption but extinction correction was applied (extinction coefficient 0.035(2)). The refinement converged to $R = 0.0359$ ($wR^2 = 0.0977$, number of variables 158, rest electron density 0.161 and −0.188 e/Å^3).

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