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TETRAHEDRON: *ASYMMETRY*

Enzymatic desymmetrization of *cis*-1,3-cyclohexanedicarboxylic acid diesters

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Abstract

cis-1,3-Cyclohexanedicarboxylic acid (1,3-CHDA) monoesters were prepared in high overall yield and high enantiomeric purity using a three step process from *cis*/*trans*-1,3-CHDA. The asymmetry is induced by an enzymatic hydrolytic desymmetrization of a *meso cis*-1,3-CHDA diester. A judicious choice of ester substituents and enzyme can provide >80% overall yield of either enantiomer in >94% ee.

Enzyme-catalyzed reactions are a powerful method for the generation of asymmetry, but are often inefficient because they are usually used to perform resolutions of racemic mixtures. Desymmetrization of *meso* compounds, where an achiral species is optimally converted to a single enantiomer of a chiral molecule, avoids the 50% yield limits and difficult separations often associated with resolutions. This is probably one of the simplest methods for the generation of an optically active material, provided that the substrate is readily available and the enzymatic reaction proceeds with sufficient enantioselectivity.

Enantioselective hydrolyses of *meso cis*-1,2-cyclohexanedicarboxylic acid diesters have been reported in a number of publications, invariably using pig liver esterase as the enzyme.[1] The desymmetrized monoester products have been used for the synthesis of both pharmaceutically active materials[2] and natural products.[3] There have also been reports of the desymmetrization of both *cis*-1,3-cyclopentanedicarboxylic anhydride (via enzymatic alcoholysis) and *cis*-1,3-cyclopentanedicarboxylic acid diesters (via enzymatic hydrolysis).[4] There have been no reports of the desymmetrization of *cis*-1,3-cyclohexanedicarboxylic acid (*cis*-1,3-CHDA) derivatives, although the enantiomerically enriched monoester products, if available, should be useful chiral synthons. For example, *cis*-3*R*-3-aminocyclohexanecarboxylic acid, a known GABA uptake inhibitor[5] and pharmaceutical building block,[6] should be readily available from the *cis*-1,3-CHDA monoester by Hofmann rearrangement of the corresponding amide.

The investigation of the enzymatic desymmetrization of a *cis*-1,3-CHDA species requires an all-*cis* precursor. Unfortunately, the parent diacid is only available as a mixture of *cis* and *trans* isomers. It is

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likely that the separation of the isomers would be troublesome, and it would certainly be more efficient if the mixture could be converted into an all-*cis* species.

Fortunately, a method was developed some years ago[7] that converted the *cis*/*trans* diacid mixture to the all-*cis* anhydride by the action of acetic anhydride on the diacid. This apparently succeeds because the *cis* arrangement is the only accessible geometry for cyclization to the anhydride and isomerization of the *trans* isomer to *cis* is feasible under the reaction conditions. Indeed, heating a mixture of *cis*- and *trans*-1,3-CHDA with acetic anhydride followed by removal of the volatiles at atmospheric pressure afforded the all-*cis* anhydride, which could be readily recrystallized to high purity in >90% yield. This cyclic anhydride appeared to be an attractive starting point for enzymatic investigation.

The enzyme-catalyzed alcoholysis of *cis*-1,3-CHDA anhydride (**1**) was the most obvious approach to an optically pure monoester. This is a type of enzymatic reaction that is most often performed in an organic solvent and is often a more flexible reaction than the opposite (hydrolysis) reaction. A fairly hindered alcohol, isopropanol, was chosen as the alcohol reactant to minimize any non-enzymatic alcoholysis (a major concern with anhydrides) that would compromise the enantiomeric purity of the product. Unfortunately, few solvents were found that would dissolve the anhydride **1** and, once dissolved, the enzymatic reactions of **1** were extremely sluggish. In addition, a survey of enzymes and solvents afforded at best only moderate enantioselectivity (maximum 78% ee as determined by chiral capillary GC) for the preparation of the monoisopropyl ester **3d**.

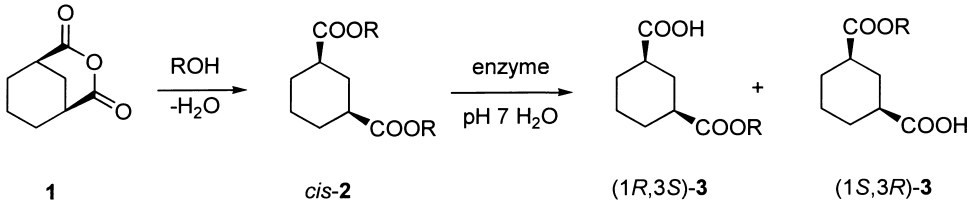
The converse reaction, enzymatic hydrolysis of a *cis*-1,3-CHDA diester, was also investigated. *cis*-1,3-CHDA anhydride (**1**) was readily converted in essentially quantitative yield to the various diesters by reaction with the desired alcohol using acid catalysis with water removal. Somewhat surprisingly, all of the diesters were oils as compared to the highly crystalline anhydride. Oils are highly desirable for enzymatic hydrolysis reactions since, unlike solid substrates, they form good emulsions in water, a requirement for a successful enzymatic hydrolysis. It was also important to note that the conversion to the diester proceeded in all cases without any observable *cis*/*trans* isomerization as determined by control reactions using *cis*/*trans*-1,3-CHDA.

In contrast to the anhydride, most of the diesters were readily hydrolyzed with a variety of enzymes to afford clean conversion to the corresponding monoester. Conveniently, none of the enzymes examined would produce the diacid even at extended reaction times. Screening a number of enzymes with a variety of *cis*-1,3-CHDA diesters demonstrated that several of these enzymatic hydrolyses proceeded with high enantioselectivity (enantiomeric excesses were determined by chiral capillary GC on a Cyclodex-B column [J&W Scientific]). A selection of these results is presented in Table 1. There are three important points to be learned from this data. First, the enzymatic hydrolyses of the diesters were much more selective than the alcoholyis of the anhydride, with several of the reactions affording enantioselectivities in the ‘useful’ range (>95% ee). Second, reactions exhibiting high but opposite (and essentially equal) enantioselectivities were observed using lipases from *Pseudomonas* species and *Candida* species, respectively. For example, Lipase PS-30 from *Pseudomonas cepacia* afforded (1*R*,3*S*)-3-alkoxycarbonylcyclohexanecarboxylic acid [(1*R*,3*S*)-**3**] while Lipase AY-30 from *Candida rugosa* afforded (1*S*,3*R*)-**3**. Thus either enantiomer of the 1,3-CHDA monoester could be obtained in high enantiomeric purity through a judicious choice of enzyme. This complementary high enantioselectivity from different commercial enzymes is quite unusual. Third, pig liver esterase, which had shown high enantioselectivity for the hydrolysis of *cis*-1,2-CHDA diesters, exhibited poor enantioselectivity in this 1,3-system.

Table 1
Enantiomeric excess[i] of *cis*-**3** from the enzymatic hydrolysis of *cis*-**2**

	Enzyme[ii]		
	PS-30	**PLE**	**AY-30**
Ester			
Me (a)	82	16	-62
Et (b)	96	34	-96
n-Pr (c)	88	32	-95
i-Pr (d)	54	32	-99
n-Bu (e)	90	47	-86

[i] defined as [%(1*R*,3*S*)-**3**] – [%(1*S*,3*R*)-**3**]
[ii] Enzyme key: **PS-30**: lipase from *Pseudomonas cepacia* (Amano International Enzyme Co.); **PLE**: pig liver esterase (Sigma); **AY-30**: lipase from *Candida rugosa* (Amano).



1 *cis*-**2** (1*R*,3*S*)-**3** (1*S*,3*R*)-**3**

a: R = Me **d**: R = i-Pr
b: R = Et **e**: R = n-Bu
c: R = n-Pr

Variation of the ester substituents can often afford a profound difference in the course of these types of reactions. In this case the enantioselectivity peaked with the diethyl ester as a substrate, falling off rapidly upon going to the dimethyl ester and gradually with increasing diester length.

A rapid reaction rate is also important for the practical application of this type of methodology. The diethyl ester was superior in this respect as well, as the reaction slowed significantly as the ester length increased. Surprisingly, the dimethyl ester exhibited lower reactivity than the diethyl ester.

The use of standard soluble enzymes for these reactions resulted in significant emulsions during product isolation, causing both operational difficulties and lower yields. Control reactions indicated that the culprit was the residual enzyme, thus removal of the enzyme prior to isolation could significantly improve the process. Enzyme immobilization on a support is a standard method to allow enzyme removal (by filtration) and has the added benefit of potentially allowing reuse of an enzyme that would normally be discarded. Commercially available crystallized, cross-linked enzymes termed ChiroCLECs™ from Altus Biologics were examined in these reactions. These systems are advantaged in that the enzyme activity and selectivity are retained and lipases from the desired two microorganisms (*Pseudomonas* and *Candida* species) are commercially available. Performing the enzymatic hydrolysis reactions with the diethyl ester under standard conditions but using the ChiroCLECs™ afforded high enantioselectivity (ChiroCLEC-PC™ [from *Pseudomonas cepacia*] gave 1*R*,3*S*-**3b** with 94% ee; ChiroCLEC-CR™ [from *Candida rugosa*] gave 1*S*,3*R*-**3b** with >95% ee) and good rates, with no emulsion observed during product isolation. Indeed, the product monoesters were obtained in nearly quantitative yield.

The absolute configuration of these *cis*-1,3-CHDA monoesters was determined by correlation with 3-methyl-1-cyclohexanemethanol, which was separable by chiral GC analysis (30 m Cyclodex-B) into all four stereoisomers. (*R*)-3-Methyl-1-(*RS*)-cyclohexanemethanol of known configuration at carbon three was prepared from (*R*)-3-methylcyclohexanone (available from Aldrich Chemical Co.) by olefination (Ph_3P=CHOMe), hydrolysis of the enol ether (aq. $HClO_4$), and reduction of the resulting (*R*)-3-methyl-1-

(*RS*)-cyclohexanecarboxaldehyde ($NaBH_4$). A single stereoisomer of 3-methyl-1-cyclohexanemethanol was prepared from monoester **3b** by reduction of the acid group to afford 3-ethoxycarbonyl-1-cyclohexanemethanol ($BH_3 \cdot SMe_2$), alcohol tosylation, and exhaustive reduction of both the tosylate and ester groups ($LiAlH_4$). Comparison of the materials prepared above by chiral GC indicated that the enantiomer prepared via Lipase PS-30 hydrolysis possessed the (*R*) configuration at carbon three of 3-methyl-1-cyclohexanemethanol (which corresponds to carbon one of compound **3b**). Thus, Lipase PS-30 hydrolysis of diethyl *cis*-1,3-CHDA afforded (1*R*,3*S*)-3-ethoxycarbonylcyclohexanecarboxylic acid [(1*R*,3*S*)-**3b**] and that derived from Lipase AY-30-catalyzed hydrolysis was the (1*S*,3*R*)-isomer. These configurational assignments should likely hold true for all of the other monoesters investigated.

With this last piece of data we now can prepare either 1,3-CHDA monoethyl ester enantiomer in high enantiomeric purity (>94% ee) and in high overall yield (>80%) with known configuration by a three-step process involving two chemical steps and one enzymatic step.

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