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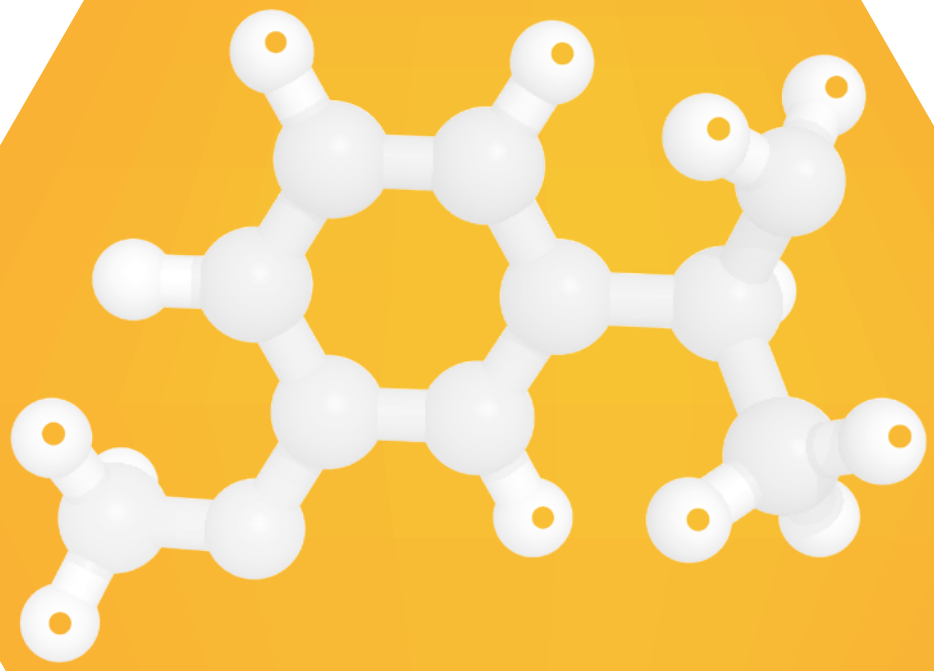

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(52) Calderini E., Süss P., Hollmann F., Wardenga R., Schallmey A. Two (chemo-)enzymatic cascades for the production of opposite enantiomers of chiral azidoalcohols (2021), doi.org/10.3390/catal11080982

Abstract: *Multi-step cascade reactions have gained increasing attention in the biocatalysis field in recent years. In particular, multi-enzymatic cascades can achieve high molecular complexity without workup of reaction intermediates thanks to the enzymes' intrinsic selectivity; and where enzymes fall short, organo- or metal catalysts can further expand the range of possible synthetic routes. Here, we present two enantiocomplementary (chemo)-enzymatic cascades composed of either a styrene monooxygenase (StyAB) or the Shi epoxidation catalyst for enantioselective alkene epoxidation in the first step, coupled with a halohydrin dehalogenase (HHDH)-catalysed regioselective epoxide ring opening in the second step for the synthesis of chiral aliphatic non-terminal azidoalcohols. Through the controlled formation of two new stereocenters, corresponding azidoalcohol products could be obtained with high regioselectivity and excellent enantioselectivity (99% ee) in the StyAB-HHDH cascade, while product enantiomeric excesses in the Shi-HHDH cascade ranged between 56 and 61%.*

(48) Ingenbosch K., Quint S., Dyllick-Brenzinger M., Wunschik D., Kiebist J., **Süss P.**, Liebelt U., Zuhse R., **Menyes U.**, Scheibner K., Mayer C., Opwis K., Gutmann J., Hoffmann-Jacobsen K.
Singlet-Oxygen Generation by Peroxidases and Peroxygenases for Chemoenzymatic Synthesis, (2020), doi.org/10.1002/cbic.202000326

Abstract: *Singlet oxygen is a reactive oxygen species undesired in living cells but a rare and valuable reagent in chemical synthesis. We present a fluorescence spectroscopic analysis of the singlet-oxygen formation activity of commercial peroxidases and novel peroxygenases. Singlet-oxygen sensor green (SOSG) is used as fluorogenic singlet oxygen trap. Establishing a kinetic model for the reaction cascade to the fluorescent SOSG endoperoxide permits a kinetic analysis of enzymatic singlet-oxygen formation. All peroxidases and peroxygenases show singlet-oxygen formation. No singlet oxygen activity could be found for any catalase under investigation. Substrate inhibition is observed for all reactive enzymes. The commercial dye-decolorizing peroxidase industrially used for dairy bleaching shows the highest singlet-oxygen activity and the lowest inhibition. This enzyme was immobilized on a textile carrier and successfully applied for a chemical synthesis. Here, ascaridole was synthesized via enzymatically produced singlet oxygen.*

(44) Sabry H., Younes H., Tieves F., Lan D., Wang Y., **Süss P., Brundiek H.**, Wever R., Hollmann F.
Chemoenzymatic Halocyclization of γ,δ-Unsaturated Carboxylic Acids and Alcohols (2019), doi.org/10.1002/cssc.201902240

Abstract: *A chemoenzymatic method for the halocyclization of unsaturated alcohols and acids by using the robust V-dependent chloroperoxidase from Curvularia inaequalis (CiVCPO) as catalyst has been developed for the in situ generation of hypohalites. A broad range of halolactones and cyclic haloethers are formed with excellent performance of the biocatalyst.*

(43) Sviatenko O., Ríos-Lombardía N., Morís F., González-Sabín J., Venkata Manideep K., Merdivan S., Günther S., **Süss P.**, Höhne M.
One-pot Synthesis of 4-Aminocyclohexanol Isomers by Combining a Keto Reductase and an Amine Transaminase (2019), DOI 10.1002/cctc.201900733

Abstract: *The efficient multifunctionalization by one-pot or cascade catalytic systems has developed as an important research field, but is often challenging due to incompatibilities or cross-reactivities of the catalysts leading to side product formation. Herein we report the stereoselective preparation of cis- and trans-4-aminocyclohexanol from the potentially bio-based precursor 1,4-cyclohexanedione. We identified regio- and stereoselective enzymes catalyzing reduction and transamination of the diketone, which can be performed in a one-pot sequential or cascade mode. For this, we identified regioselective keto reductases for the selective mono reduction of the diketone to give 4-hydroxycyclohexanone. The system is modular and by choosing stereocomplementary amine transaminases, both cis- and trans-4-aminocyclohexanol were synthesized with good to excellent diastereomeric ratios. Furthermore, we identified an amine transaminase that produces cis-1,4-cyclohexanediamine with diastereomeric ratios >98 : 2. These examples highlight that the high selectivity of enzymes enable short and stereoselective cascade multifunctionalizations to generate high-value building blocks from renewable starting materials.*

(42) Marx L., Rios-Lombardia N., **Süss P.**, Höhne M., Moris F., Gonzales-Sabin J., Berglund P.
Chemoenzymatic Synthesis of Sertraline (2019), DOI 10.1002/ejoc.201901810

Abstract: *A chemoenzymatic approach has been developed for the preparation of sertraline, an established anti-depressant drug. Ketoreductases (KREDs) were employed to yield a key chiral precursor. The bioreduction of the racemic tetralone exhibited excellent enantioselectivity (>99% ee) and diastereomeric ratio (99:1) at 29% conversion (the maximum theoretical yield is 50%) after 7 hours. The resulting (S,S)-alcohol was efficiently oxidized to an enantiopure (S)-ketone, an immediate precursor of sertraline, by using sodium hypochlorite as oxidant and 2-azaadamantane N-oxyl (AZADO) as organocatalyst. Alternative routes aiming at the direct biocatatalytic amination using imine reductases and transaminases were unsuccessful.*

(41) *Calderini E., Wessel J.,* **Süss P.**, *Schrepfer P.,* **Wardenga R.**, *Schallmey A.*
Selective Ring-Opening of Di-substituted Epoxides Catalysed by Halohydrin Dehalogenases; ChemCatChem (2019), DOI 10.1002/cctc.201900103

Abstract: *Halohydrin dehalogenases (HHDHs) are valuable biocatalysts for the synthesis of β-substituted alcohols based on their epoxide ring-opening activity with a number of small anionic nucleophiles. In an attempt to further broaden the scope of substrates accepted by these enzymes, a panel of 22 HHDHs was investigated in the conversion of aliphatic and aromatic vicinally di-substituted trans-epoxides using azide as nucleophile. The majority of these HHDHs was able to convert aliphatic methyl-substituted epoxide substrates to the corresponding azido-alcohols, in some cases even with absolute regioselectivity. HheG from Ilumatobacter coccineus exhibited also high activity towards sterically more demanding di-substituted epoxides. This further expands the range of β-substituted alcohols that are accessible by HHDH catalysis.*

(40) Zhang W., Fernandez Fueyo E.F., Hollmann F., Leemans-Martin L., Pesic M., **Wardenga R.**, Höhne M., Schmidt S., (2018)
Combining Photo-Organo Redox- and Enzyme Catalysis Facilitates Asymmetric C-H Bond Functionalization, European Journal of Organic Chemistry DOI: 10.1002/ejoc.201801692

Abstract: *In this study, we combined photo-organo redox catalysis and biocatalysis to achieve asymmetric C–H bond functionalization of simple alkane starting materials. The photocatalyst anthraquinone sulfate (SAS) was employed to oxyfunctionalise alkanes to aldehydes and ketones. We coupled this light-driven reaction with asymmetric enzymatic functionalisations to yield chiral hydroxynitriles, amines, acyloins and α-chiral ketones with up to 99% ee. In addition, we demonstrate functional group interconversion to alcohols, esters and carboxylic acids. The transformations can be performed as concurrent tandem reactions. We identified the degradation of substrates and inhibition of the biocatalysts as limiting factors affecting compatibility, due to reactive oxygen species generated in the photocatalytic step. These incompatibilities were addressed by reaction engineering such as, applying a two-phase system or temporal and spatial separation of the catalysts. Using a selection of eleven starting alkanes, one photo-organo catalyst and 8 diverse biocatalysts, we synthesized 26 products and report for the model compounds benzoin and mandelonitrile >97% ee at gram scale.*

(39) Meissner M.P., **Süss P., Brundiek H.**, Woodley J.M., v. Langermann J.
Scoping the Enantioselective Desymmetrization of a Poorly Water-Soluble Diester by Recombinant Pig Liver Esterase, Org. Process Res. Dev., Just Accepted Manuscript, Org. Process Res. Dev. 2018, 22, 1518–1523.

Abstract: *Previously, the biocatalytic desymmetrization of dimethyl cyclohex-4-ene-cis-1,2-dicarboxylate to (1S,2R)-1-(methoxycarbonyl)cyclohex-4-ene-2-carboxylic acid, an important intermediate towards the synthesis of biologically active molecules, had been well-characterized in terms of pH and temperature optima and several aspects of process performance. Eventually this promising reaction could convert 200 mM (40 g·L-1) of substrate with > 99.5% e.e. using the recombinant pig liver esterase, ECS-PLE06, at a scale of 8.8 L. However, the precise influence of substrate concentration and the poorly water-soluble nature of the substrate (approximately 60 mM in water at 25 °C for structurally similar dimethyl 1,4-cyclohexanedicarboxylate) remained elusive. Therefore, this work focuses on using a recently published methodology based on reaction trajectory analysis to identify mass transfer limitations in this reaction. With the constraints of mass transfer on space-time yield considered, it was possible to evaluate and improve biocatalyst yield (mass of product per mass of biocatalyst) through the use of higher substrate concentrations. Ultimately the complete conversion of approximately 75 g·L-1 substrate was achieved in 3.65 h yielding an excellent productivity of 20 g·L-1·h-1 with a biocatalyst yield of 4.36 g·g¬¬biocat-1. This work also highlights the simplicity of applying a reaction trajectory analysis methodology, importance of scale during reaction characterizations and identifies future directions for reaction improvement to address substrate supply and product inhibition/deactivation.*

(36) Schoenenberger B., **Wszolek A.**, Meier R., **Brundiek H.**, Obkircher M., Wohlgemuth R. (2018).
Recombinant AroL-catalyzed Phosphorylation for the efficient Synthesis of Shikimic acid 3-phosphate. Biotechnology journal, 1700529/biot.201700529

Abstract: *Shikimic acid 3-phosphate, as a central metabolite of the shikimate pathway, is of high interest as enzyme substrate for 5-enolpyruvoyl-shikimate 3-phosphate synthase, a drug target in infectious diseases and a prime enzyme target for the herbicide glyphosate. As the important substrate shikimic acid 3-phosphate is only accessible via a chemical multi-step route, a new straightforward preparative one-step enzymatic phosphorylation of shikimate using a stable recombinant shikimate kinase has been developed for the selective phosphorylation of shikimate in the 3-position. Highly active shikimate kinase is produced by straightforward expression of a synthetic aroL gene in Escherichia coli. The time course of the shikimate kinase-catalyzed phosphorylation is investigated by 1H- and 31P-NMR, using the phosphoenolpyruvate/pyruvate kinase system for the regeneration of the ATP cofactor. This enables the development of a quantitative biocatalytic 3-phosphorylation of shikimic acid. After a standard workup procedure, a good yield of shikimic acid 3-phosphate, with high HPLC- and NMR purity, is obtained. This efficient biocatalytic synthesis of shikimic acid 3-phosphate is superior to any other method and has been successfully scaled up to multi-gram scale.*

(34) R. Wardenga (2017)
Biocatalytic access to a novel class of Mannich catalysts; Manufacturing Chemist, October 2017, pp 52-53

Abstract: *Enzymicals AG highlights the possibility to synthesize novel diastereomers of 5-benzyl-C□-methyl-□-proline by stereoselective hydrolysis of branched malonate diesters. Application of recombinant pig liver esterase isoenzymes was the key to success to gain the (3S,5S)-diastereomer which shows activity as anti-Mannich catalyst.*

(33) B. Schoenenberger, **A. Wszolek**, R. Meier, **H. Brundiek**, M. Obkircher,and R. Wohlgemuth (2017),
Biocatalytic asymmetric Michael addition reaction of L-arginine to fumarate for the green synthesis of N-(([(4S)-4-amino-4-carboxy-butyl]amino) iminomethyl)-L-aspartic acid lithium salt (L-argininosuccinic acid lithium salt) RCS-Advances, 2017, 7, 48952; DOI: 10.1039/c7ra10236d

Abstract: *The basic natural amino acid L-argininosuccinate containing two chiral centers occurs in L-alanine, L-arginine, L-aspartate, L-glutamate and L-proline metabolic pathways and plays a role in the biosynthesis of secondary metabolites and other amino acids. It is a precursor for arginine in the urea cycle or the citrulline–NO cycle as well as a precursor to fumarate in the citric acid cycle via argininosuccinate lyase. We aimed to run part of the urea cycle in reverse by catalyzing not the elimination but the addition reaction of L-arginine to fumarate in order to synthesize L-argininosuccinate. Argininosuccinate lyase (ASL) from Saccharomyces cerevisiae has been chosen as the catalyst for this addition reaction. The selected ARG4 gene was synthesized and homogeneously expressed in E. coli leading to a highly active argininosuccinate lyase. The ASL-catalyzed addition reaction of L-arginine to fumarate has been successfully developed at gram scale. After a standard workup procedure the pure final product L-argininosuccinate has been isolated in good yield and high purity.*

(32) Kotapati H.K., Robinson J., Lawrence D., Fortner K., Stanford C., Powell D., **Wardenga R.**, Bornscheuer U. (2017),
Diastereoselective hydrolysis of branched malonate diesters by Porcine Liver Esterase: Synthesis of 5-benzyl substituted Cα-methyl-β-proline and catalytic evaluation, European Journal of Organic Chemistry, DOI: 10.1002/ejoc.201700605

Abstract: *Malonate diesters with highly branched side chains containing a preexisting chiral center were prepared from optically pure amino alcohols and subjected to asymmetric enzymatic hydrolysis by Porcine Liver Esterase (PLE). Recombinant PLE isoenzymes have been utilized in this work to synthesize diastereomerically enriched malonate half-esters from enantiopure malonate diesters. The diastereomeric excess of the product half-esters was further improved in the later steps of synthesis either by simple recrystallization or flash column chromatography. The diastereomerically enriched half-ester was transformed into a novel 5-substituted Cα-methyl-β-proline analogue (3R, 5S)-1c, in high optical purity employing a stereoselective cyclization methodology. This β-proline analogue was tested for activity as a catalyst of the Mannich reaction. The β-proline analogue derived from the hydrolysis reaction by the crude PLE appeared to catalyze the Mannich reaction between an α-imino ester and an aldehyde providing decent to good diastereoselectivities. However, the enantioselectivities in the reaction was low. The second diastereomer of the 5-benzyl substituted Cα-methyl-β-proline, (3S, 5S)-1c was prepared by enzymatic hydrolysis using PLE isoenzyme 3 and tested for its catalytic activity in the Mannich reaction. Amino acid, (3S, 5S)-1c catalyzed the Mannich reaction between isovaleraldehyde and an α-imino ester yielding the „anti" selective product with an optical purity of 99%ee.*

(30) Schoenenberger B., **Wszolek A.**, Milesi T., Obkircher M., **Brundiek H.**, Wohlgemuth R. (2016),
Synthesis of Nω-Phospho-L-arginine by Biocatalytic Phosphorylation of L-Arginine, Chemcatchem, 10.1002/cctc.201601080, Volume 9, Issue 1, January 9, 2017, Pages 121–126

Abstract: *The Nω-Phospho-L-arginine energy-buffering system is mainly present in invertebrates for regulating energy requirements when it is highly needed, as in the flight muscle of an insect or when energy supply fluctuates, as in the medically important protozoa Trypanosoma brucei, Trypanosoma cruzi and Leishmania major. The lacking availability of this important metabolite was due to a tedious chemical procedure, by which Nω-phospho-L-arginine was prepared up to now over 5 reaction steps in a low yield. Therefore, we aimed at improving the synthetic methodology for the preparation of this important metabolite. As site- and enantioselective kinases have been very useful catalysts for biocatalytic phosphorylations in straightforward syntheses of phosphorylated metabolites, a stable and selective arginine kinase has been selected for the selective phosphorylation of L-arginine. The arg gene has been cloned and expressed in E.coli and a highly active arginine kinase has been prepared. A simple synthesis of Nω-phospho-L-arginine has been developed by arginine kinase-catalyzed phosphorylation of L-arginine combined with the recycling of the phosphorylating agent ATP using the phosphoenolpyruvate/pyruvate kinase system. After standard workup the desired product Nω-Phospho-L-arginine has been obtained in gram quantities and in one step.*

(23) Kohls H., Sowa M., Anderson M., Dickerhoff J., Weisz K., Córdova A.; Berglund P., Bornscheuer U.T., **Brundiek H.**, Höhne M. (2015)
Selective Access to All Four Diastereomers of a 1,3-Amino Alcohol by Combination of a Keto Reductase- and an Amine Transaminase-Catalysed Reaction, Advanced Synthesis & Catalysis, 357 (8), 1808–1814

Abstract: *The biocatalytic synthesis of chiral amines has become a valuable addition to the chemists' toolbox. However, the efficient asymmetric synthesis of functionalised amines bearing more than one stereocentre, such as 1,3-amino alcohols, remains challenging. By employing a keto reductase (KRED) and two enantiocomplementary amine transaminases (ATA), we developed a biocatalytic route towards all four diastereomers of 4-amino-1-phenylpentane-2-ol as a representative molecule bearing the 1,3-amino alcohol functionality. Starting from a racemic hydroxy ketone, a kinetic resolution using an (S)-selective KRED provided optically active hydroxy ketone (86% ee) and the corresponding diketone. Further transamination of the hydroxy ketone was performed by either an (R)- or an (S)-selective ATA, yielding the (2R,4R)- and (2R,4S)-1,3-amino alcohol diastereomers. The remaining two diastereomers were accessible in two subsequent asymmetric steps: the diketone was reduced regio- and enantioselectively by the same KRED, which yielded the (S)-configured hydroxy ketone. Eventually, the subsequent transamination of the crude product with (R)- and (S)-selective ATAs yielded the remaining (2S,4R)- and (2S,4S)-diastereomers, respectively.*

(16) Sehl, T., Hailes, C. H., Ward, J. M., **Menyes, U.**, Pohl, M., Rother, D. (2014)
Efficient 2-step biocatalytic strategies for the synthesis of all nor(pseudo)ephedrine isomers. Green Chem., 2014, 16, 3341-3348

Abstract: *Chiral 1,2-amino alcohols are important building blocks for chemistry and pharmacy. Here, we developed two different biocatalytic 2-step cascades for the synthesis of all four nor(pseudo)ephedrine (N(P)E) stereoisomers. In the first one, the combination of an (R)-selective thiamine diphosphate (ThDP)-dependent carboligase with an (S)- or (R)-selective ω-transaminase resulted in the formation of (1R,2S)-NE or (1R,2R)-NPE in excellent optical purities (ee >99% and de >98%). For the synthesis of (1R,2R)-NPE, space–time yields up to 26 g L–1 d–1 have been achieved. Since a highly (S)-selective carboligase is currently not available for this reaction, another strategy was followed to complement the nor(pseudo)ephedrine platform. Here, the combination of an (S)-selective transaminase with an (S)-selective alcohol dehydrogenase yielded (1S,2S)-NPE with an ee >98% and a de >99%. Although lyophilized whole cells are cheap to prepare and were shown to be appropriate for use as biocatalysts, higher optical purities were observed with purified enzymes. These synthetic enzyme cascade reactions render the N(P)E-products accessible from inexpensive, achiral starting materials in only two reaction steps and without the isolation of the reaction intermediates.*

(14) Wardenga, R., Bednarczyk, A., Höhne, M. (2013),
Asymmetric synthesis of chiral amines from ketones. How to apply biocatalysis and find a suitable enzyme. PharmaChem, 12, 22-25.

Abstract: *Optically active amines play an important role in the pharmaceutical, agrochemical, and chemical industries. They are frequently used as synthons for the preparation of various pharmaceutically active substances. Consequently, there is a need for efficient methods to obtain the desired enantiomer of a given target structure in an optically pure form. Beside a range of chemical methods using for example, asymmetric synthesis with transition metal catalysts, enzymes represent a useful alternative to access this important class of compounds. This article focusses on the biocatalytic transaminase approach with emphasis on how to screen for suitable catalysts for the asymmetric synthesis starting from prostereogenic ketones.*

(13) Sehl, T., Hailes, H. C., Ward, J. M., **Wardenga, R.**, von Lieres, E., Offermann, H., Westphal, R., Pohl, M., Rother, D., (2013)
Two Steps in One Pot: Enzyme Cascade for the Synthesis of Nor(pseudo)ephedrine from Inexpensive Starting Materials. Angewandte Chemie International Edition vol. 52 (26) 6772-6775.

***Abstract**: Keywords: asymmetric synthesis; biocatalysis; enzyme cascades; phenylpropanolamine; ω-transaminase*
Two steps in one pot: An enzyme cascade consisting of a lyase and an (R)- or (S)-selective ω-transaminase (TA) provides (1R,2R)-norpseudoephedrine and (1R,2S)-norephedrine in only two steps. The intermediate is not isolated in this one-pot reaction and the products are obtained in high enantio- and diastereomeric purity. Moreover, the by-product from the second reaction can be recycled to serve as the substrate for the first reaction.

(12) Staudt, S., Bornscheuer, U.T., **Menyes, U.**, Hummel, W., Gröger, H. (2013),
Direct biocatalytic one-pot-transformation of cyclohexanol with molecular oxygen into ε-caprolactone, Enzyme Microb. Technol., 53, 288-292.

Abstract: The development of a biocatalytic process concept for ε-caprolactone, which directly converts cyclohexanol as an easily available industrial raw material into the desired ε-caprolactone in a one-pot fashion while only requiring air as sole reagent, is reported. The desired product ε-caprolactone was obtained with 94–97% conversion when operating at a substrate concentration in the range of 20–60 mM. At higher substrate concentrations, however, a significant drop of conversion was found. Subsequent detailed studies on the impact of the starting material, intermediate and product components revealed a significant inhibition and partial deactivation of the BVMO by the product ε-caprolactone (in particular at higher concentrations) as well as an inhibition of the BVMO by cyclohexanol and cyclohexanone.

(10) Smith, M.E., Banerjee, S., Shi, Y., **Schmidt, M.**, Bornscheuer, U.T. Masterson, D.S. (2012),
Investigation of the cosolvent effect on six isoenzymes of PLE in the enantioselective hydrolysis of selected a,a-disubstituted malonate esters, ChemCatChem, 4, 472-475

***Abstract:** Keywords: cosolvent effects; enantioselectivity; enzymes; inversion of chirality; synthesis*
Six pigs in a pot: Pig liver esterase (PLE) is among the most widely studied esterase enzymes utilized in organic synthesis. Here we illustrate that the six recombinantly produced isoenzymes of PLE exhibit varying enantioselectivity during the hydrolysis of α,α-disubstituted malonate esters in cosolvent mixtures. We have observed a rare cosolvent-induced reversal of enantioselectivity for isoenzyme PLE 6 in the hydrolysis of a phthalimide-containing α,α-disubstituted malonate ester.