# 18.6. Catalyzed Resolution and Simultaneous Selective Crystallization

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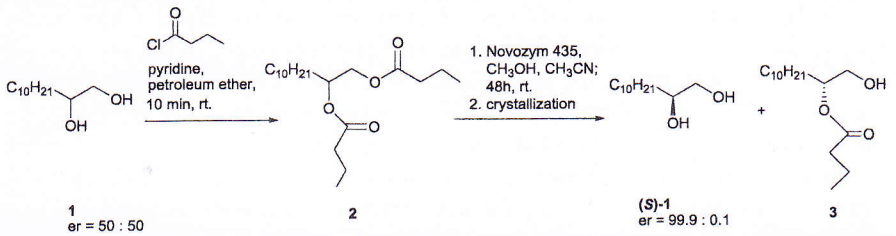


| Number of sessions (duration of each session) | Hazard level | Difficulty level | Level of study |
|---|---|---|---|
| 2 (4 h each) | Moderate | Medium | Intermediate |

**Class names** Alcohols, esters, acid chloride

**Concepts involved** This experiment involves a prompt separation of (*S*)-1,2-dodecanediol from a racemic mixture of enantiomers, *via* lipase-catalyzed methanolysis of crude chemically prepared diester, followed by *in situ* crystallization of the pure enantiomer from the reaction mixture

**Chemicals needed** 1,2-Dodecanediol (racemic), butyryl chloride, pyridine, petroleum ether (40–60 °C) (PE), methanol, $H_2O$, $NaHCO_3$, NaCl, $MgSO_4$, acetonitrile, Novozym® 435 (*Candida antarctica* lipase B immobilized on a solid carrier), ethyl acetate (EtOAc), thin-layer chromatography (TLC) visualization reagent (EtOH/$H_2SO_4$/anisaldehyde 90/10/3)

**Equipment and experimental techniques involved** Apparatuses for (1) reaction with magnetic stirring, (2) liquid–liquid extraction, and (3) for filtering through paper filter; rotary evaporator, precoated silica gel 60 $F_{254}$ plates for TLC; a removable paper bag (*e.g.* an empty "teabag" or a bag made from filter paper) filled with Novozym® 435; crystallization; apparatuses for vacuum filtering through glass filter and for determination of melting point, polarimeter

**Keywords** Crystallization, (*S*)-1,2-dodecanediol, kinetic resolution, lipase-catalyzed methanolysis, specific rotation

## Background

Single pure enantiomers of 1,2-dodecanediol (**1**) are valuable chiral building blocks for several synthetic applications in the field of biologically active compounds.[1] Pure stereoisomers of alkanediols and alkanetetrols of this type can be prepared by chemical hydrolytic kinetic resolution of terminal epoxides using Jacobsen's catalyst.[2–4] Methods of asymmetric synthesis, such as asymmetric dihydroxylation of olefins[5] or diboration–oxidation of olefins and alkynes[6] can be used as well.

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Starting from racemic 1,2-alkanediols, lipase-catalytic kinetic resolution based on stereoselective acyl transfer reactions has found wide application.[7–12]

In general, enzymes are known to work best by catalyzing reactions of their natural substrates and in their natural environment – water. However, several lipases tolerate artificial substrates and are catalytically very active also in organic solvents.[13] The enzyme preparation Novozym® 435 used in this protocol includes the lipase B from the microorganism *Candida antarctica* immobilized on the solid carrier levatite.

The idea behind the enzyme-catalyzed kinetic resolution of enantiomers is that the enzyme distinguishes between enantiomers because of their different 3D structure. The reaction catalyzed by the enzyme occurs much faster with one enantiomer than the other – and, thus, it is possible to stop the reaction at a suitable point of conversion to afford a kinetically resolved enantiomeric product with the desired enantiomeric ratio (er) value. In the current experiment, spontaneous *in situ* crystallization of (*S*)-1,2-dodecanediol (produced by the lipase-catalyzed methanolysis of butyric diester) from the reaction mixture is stereo- and chemoselective – providing the product in high chemical and stereochemical purity.[12]

Thus, the goal of the current experiment is to demonstrate a prompt, simple method, *e.g.* without using column chromatography, for separation of the crystalline (*S*)-enantiomer of 1,2-dodecanediol from a racemic mixture that allows us to gain the product with very high enantiomeric purity (er > 99.8/0.2).

## Additional Safety

All steps should be carried out in a fume hood using personal protective equipment (protective clothing, gloves, and safety goggles). Butyryl chloride is flammable, corrosive, reacts violently with water, and may cause skin and eye burns. Pyridine is flammable and toxic. Petroleum ether is flammable. Methanol is both flammable and toxic.

## Experimental Procedure

### Laboratory Session 1 (4 h)

#### *Chemical Acylation of 1,2-Dodecane Diol*

1. Prepare a 100 mL round-bottomed flask with a magnetic stirring bar, then add 1 g (5 mmol, 1 equiv.) of racemic 1,2-dodecanediol (**1**). After that, add 4 mL of pyridine, with gentle stirring and warming until the diol has dissolved.
2. Add 12 mL of PE and stir until the solution is homogeneous.
3. Add dropwise 1.3 mL (12 mmol, 2.4 equiv.) of butyryl chloride while ensuring effective stirring of the forming heterogeneous solution. Let the reaction stir for 15 min.
4. Check the completion of the reaction by performing TLC, comparing the reaction mixture with the starting 1,2-dodecanediol (**1**). Eluent for TLC: mixture of EtOAc (3 mL) and PE (5 mL). Visualization of TLC stains with anisaldehyde reagent on heating.
5. If the reaction has completed, finish the synthesis by adding methanol (0.5 mL, 12.3 mmol) with the aim of eliminating excess butyryl chloride. Stir for an additional 10 min.
6. Then add 40 mL of PE into the flask followed by 15 mL of 10% $NaHCO_3/H_2O$ solution. The latter should be added carefully due to possible intense gas evolution.
7. Transfer the reaction mixture into a separatory funnel (250 mL), add an additional portion of PE (40 mL), shake the mixture and separate the water layer.
8. Wash the organic layer once with aqueous $NaHCO_3$ solution and twice with brine (2 × 15 mL). Dry over $MgSO_4$ for 15 min.
9. Filter the drying agent from the solution and evaporate the solvents. The obtained crude product of diester **2** is used in the following enzymatic reaction without purification.

### *Enzymatic Separation of Enantiomers* via *Methanolysis Complemented by* In situ *Crystallization*

10. Dissolve the crude diester **2** in acetonitrile (9.6 mL), add methanol (0.4 mL), introduce Novozym 435® (0.5 g) packed in a removable paper bag and a small stirring bar into the reaction flask. Stir on a magnetic stirrer for at least 48 h.

## Laboratory Session 2 (4 h)

1. Check the conversion rate of the reaction by performing TLC, comparing the reaction mixture with 1,2-dodecanediol (**1**) and diester **2**. Eluent for TLC: mixture of EtOAc (3 mL) and PE (5 mL). Visualization of TLC stains with anisaldehyde reagent. By TLC, the reaction mixture should contain only trace amounts of the starting diester along with a mixture of comparable amounts of 1,2-dodecanediol (**1**) and its 2-monoester (**3**). The crystalline product should have become observable with the naked eye.

### *Isolation and Characterization of the Product*

2. Stop the reaction by removing the enzyme bag (the bag should be carefully washed with 15 mL of EtOAc in a separate vessel and the washing added later to the crude product/mother liquor from which sequential crystallization of an additional portion of highly pure (*S*)-diol can be performed; see the next procedure).
3. Now filter out the crystalline product using a glass filter. Approximately 100 mg of the product crystals are gained (yield: 10%). The residual crude product/mother liquor (with added washing of the enzyme bag) can be recrystallized from chloroform (dissolve in 2 mL on heating, then stir briefly on an ice bath and filter the product) in order to gain an additional portion of the pure (*S*)-diol and to increase the yield up to 29%.[12]
4. The crystalline product should be dried in a vacuum desiccator for 15 min in order to separate solvent residues. The product should be weighed, the yield calculated, and the homogeneity of the product determined by TLC analysis.
5. Measure the optical rotation of the obtained product in EtOH and calculate the specific rotation.
6. Measure the melting point of the obtained pure enantiomer as well as of the racemic 1,2-dodecanediol.

## Waste Disposal

The aqueous solution from liquid–liquid extraction should be collected into a special container of waste water. The solvents evaporated and condensed in a rotary evaporator should be collected and disposed of in a non-chlorinated waste container.

## Results Interpretation and Additional Questions

1. Compare the specific rotation value determined for the separated enantiomer with the literature data and, based on this data, calculate the enantiomeric excess (ee) as well as the enantiomeric ratio (er), of the product.
2. Compare the melting point of the separated (*S*)-enantiomer with that of the racemic sample as well as with corresponding literature data; explain the results.[12]
3. Calculate the yield of crystalline (*S*)-1,2-dodecanediol obtained from the racemic material.
4. Explain the mechanism of the lipase-catalyzed sequential methanolysis used for resolution of the enantiomers.

## References


1. C. A. Challenger, *Chiral Intermediates*, John Wiley & Sons, London, 2001.
2. M. Tokunaga, J. F. Larrow, F. Kakiuchi and E. N. Jacobsen, *Science*, 1997, **277**, 936.
3. S. Chow and W. Kitching, *Chem. Commun.*, 2001, 1040.
4. J. Bredihhina, P. Villo, K. Andersons, L. Toom and L. Vares, *J. Org. Chem.*, 2013, **78**, 2379.
5. H. C. Kolb, M. S. Van Nieuwenhze and K. B. Sharpless, *Chem. Rev.*, 1994, **94**, 2483.
6. Y. Lee, H. Jang and A. H. Hoveyda, *J. Am. Chem. Soc.*, 2009, **131**, 18234.
7. Y. Shimada, H. Sato, S. Minowa and K. Matsumoto, *Synlett*, 2008, **3**, 367.
8. M.-J. Kim and Y. K. Choi, *J. Org. Chem.*, 1992, **57**, 1605.
9. L. Poppe, L. Novák, M. Kajtár-Peredy and C. Szántay, *Tetrahedron: Asymmetry*, 1993, **4**, 2211.
10. E. Santaniello, S. Casati, P. Ciuffreda and L. Gamberoni, *Tetrahedron: Asymmetry*, 2005, **16**, 1705.
11. P. Virsu, A. Liljeblad, A. Kanerva and L. T. Kanerva, *Tetrahedron: Asymmetry*, 2001, **12**, 2447.
12. O. Parve, I. Reile, J. Parve, S. Kasvandik, M. Kudrjashova, S. Tamp, A. Metsala, L. Villo, T. Pehk, J. Jarvet and L. Vares, *J. Org. Chem.*, 2013, **78**, 12795.
13. I. Vallikivi, Ü. Lille, A. Lookene, A. Metsala, P. Sikk, V. Tõugu, H. Vija, L. Villo and O. Parve, *J. Mol. Catal. B: Enzym.*, 2003, **22**, 279.