

enantis, s.r.o.
BRNO, CZECH REPUBLIC

practice 4:

BIOCATALYTIC PREPARATION OF PHARMACEUTICAL PRECURSOR (S)-2-BROMOPENTANE

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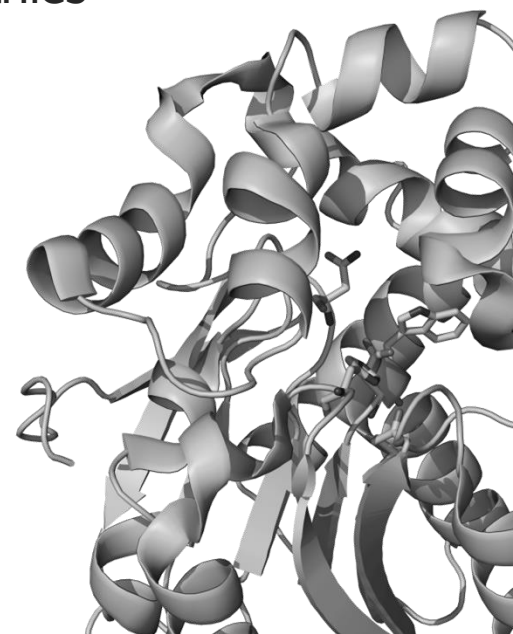
enantis profile

- the first **biotech spin-off** of Masaryk University
- tight cooperation with the **Loschmidt Laboratories**
- **consulting and development services** in the field of enzyme technologies and protein engineering
- **own products** based on dehalogenase enzymes



haloalkane dehalogenases

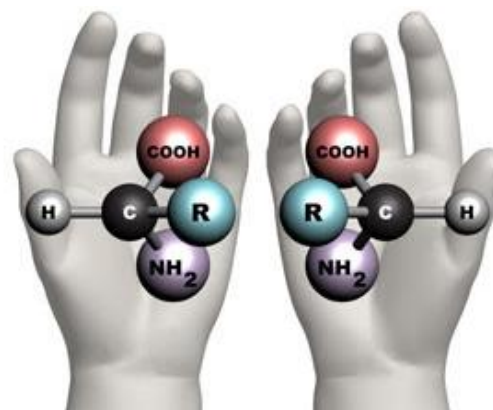
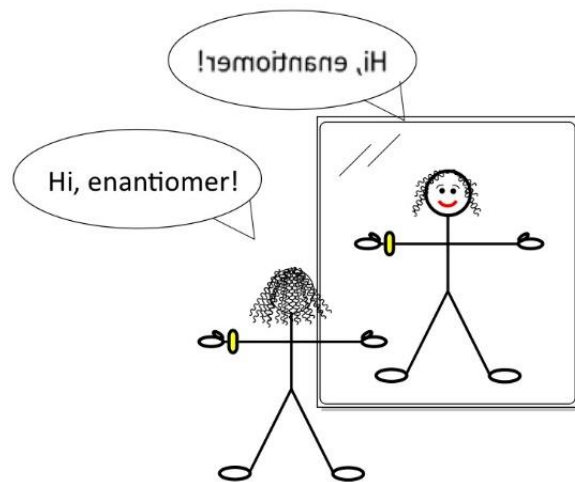
- cleavage of a **carbon-halogen** bond
- broad **substrate specificity**
 - haloalkanes, haloalkenes, cyclohaloalkanes, haloalcohols, halohydrins, haloethers, haloesters, haloamides and haloacetonitriles
- high **enantioselectivity**
 - production of optically pure haloalkanes, alcohols, halopolyols and polyols
- **no cofactors**



optical activity

- **chiral molecule**

- has two enantiomeric forms
- does not have a plane of symmetry
- is not superposable with its mirror image



optical activity

- **enantiomers**

- may have substantially different biological effects!!!

THALIDOMIDE

(*R*)-enantiomer
sedative
or hypnotic



(*S*)-enantiomer
teratogenic
and carcinogen

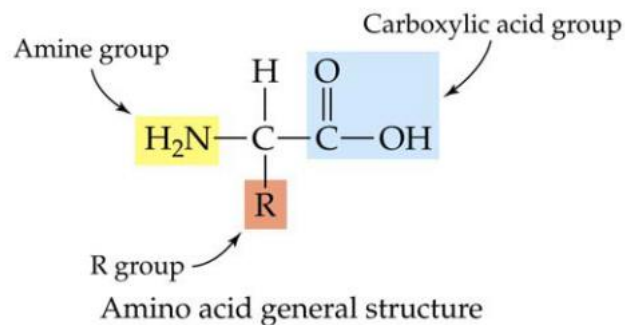
**high optical purity (> 97 %) required in pharmaceutical
and agrochemical industry**

synthesis of optically pure compounds

- chemical

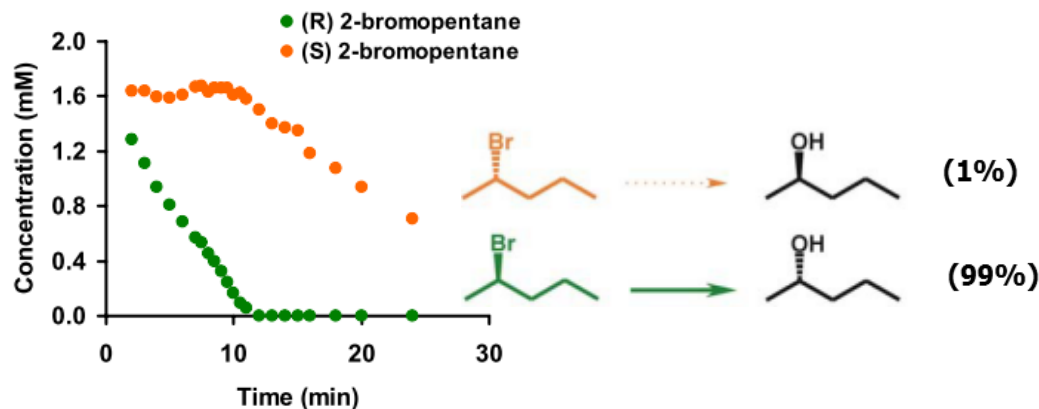
synthesis of optically pure compounds

- chemical
- **enzymatical**
 - enzymes are chiral biomolecules made from L-amino acids
 - enzymes recognize chirality of substrate (**kinetic resolution**)
 - enzymes are **enantioselective**



kinetic resolution

- theoretical maximum yield - 50%
- **enantiomeric ratio** (E) - ratio of specificity constants
 - E-value = 1 - enzyme is not stereoselective
- **enantiomeric excess** (e.e.) - enantiomeric purity



$$E = \frac{k_A}{k_B} = \frac{(k_{\text{cat}} / K_m)_R}{(k_{\text{cat}} / K_m)_S}$$

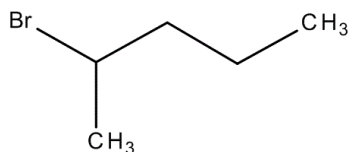
$$\text{e.e.} = \left| \frac{C_R - C_S}{C_R + C_S} \right|$$

practice 4

▪ workflow

- preparation of reaction mixtures
- enantioselectivity measurement
- gas chromatography analysis
- calculation of enantiomeric excess and yield

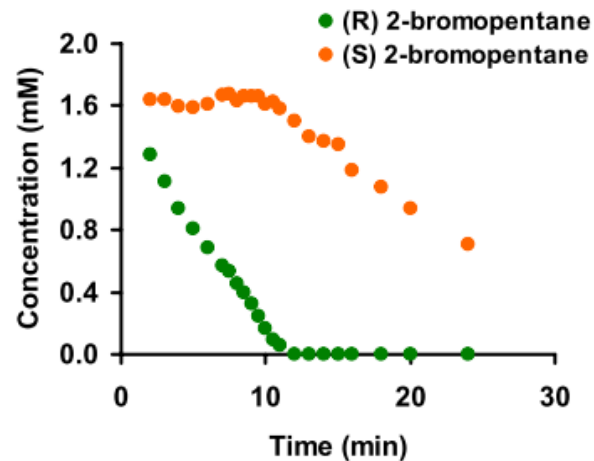
▪ protocol



substrate
2-bromopentane



biocatalyst
haloalkane dehalogenase DbjA



contact

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