About OMICS Group

OMICS Group International is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 400 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 300 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

About OMICS Group Conferences

OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Phrama scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

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In situ NMR and STD NMR to Study Mechanisms of Enzyme Catalyzed Reactions to Optimize Use of Enzymes in Organic Synthesis

Lothar Brecker

International Summit on Past and Present
Research Systems of Green Chemistry

Philadelphia (Pennsylvania, USA), August 25th – 27th 2014

Modern Synthesis in Green Chemistry

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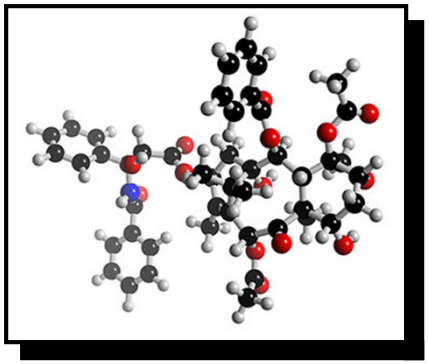
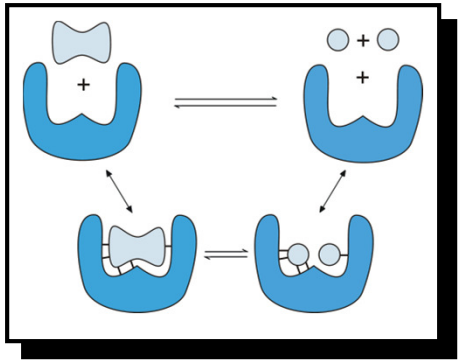
... is used to synthesize complex molecules.



The syntheses often include

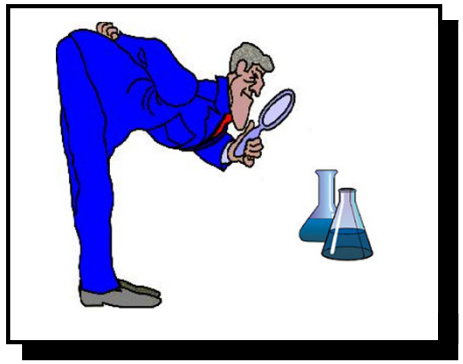
- new chemical reactions
- biocatalyzed transformations



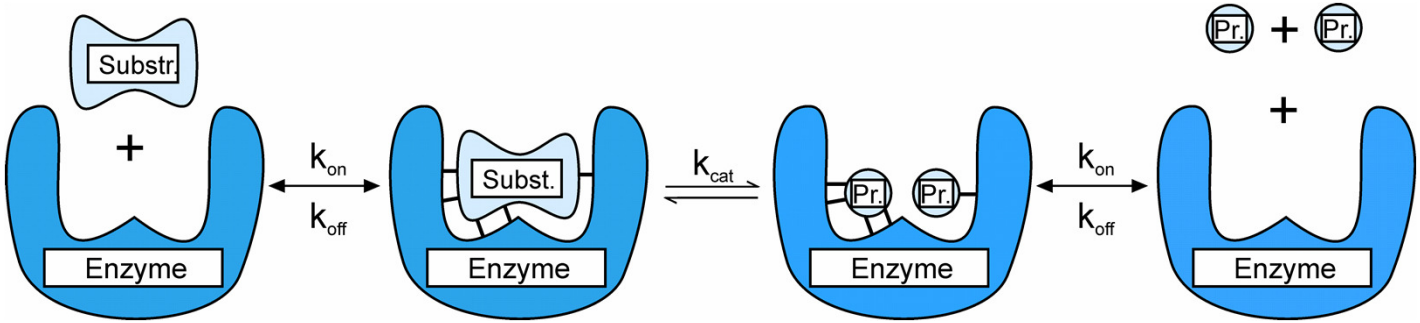
An effective application requires

knowledge about details of the reactions,

in particular in the field of biocatalysis.



Details of Biocatalyzed Reactions



- Solutions structure of starting materials
- Progress of the reaction and reaction rate
- Kinetic data
- Parallel and consecutive reactions as well as metabolisms
- Identification of intermediates
- Substrate and product binding to enzymes
- Reaction mechanisms
- Structure of products

NMR as Analytical Method

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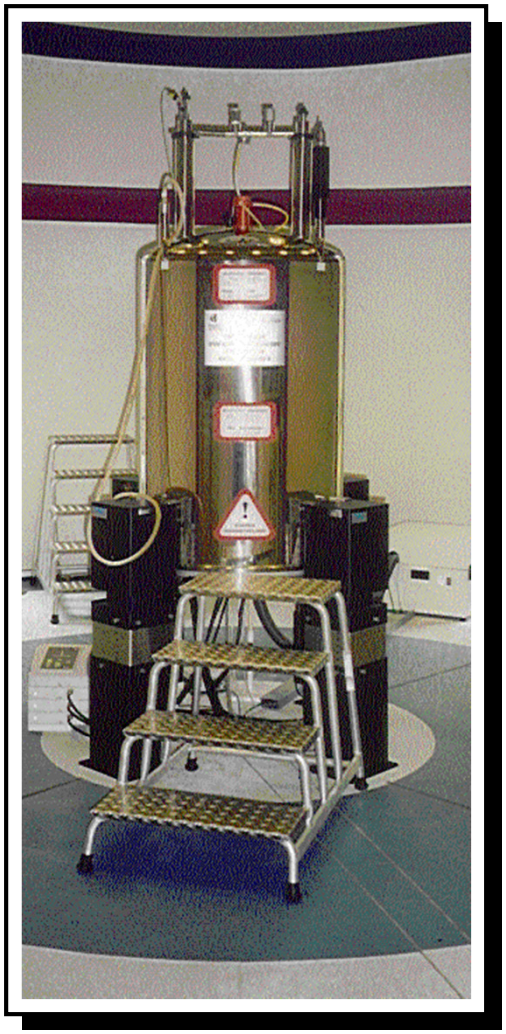
"NMR Spectroscopy is a window to the world of biocatalysed reactions!"

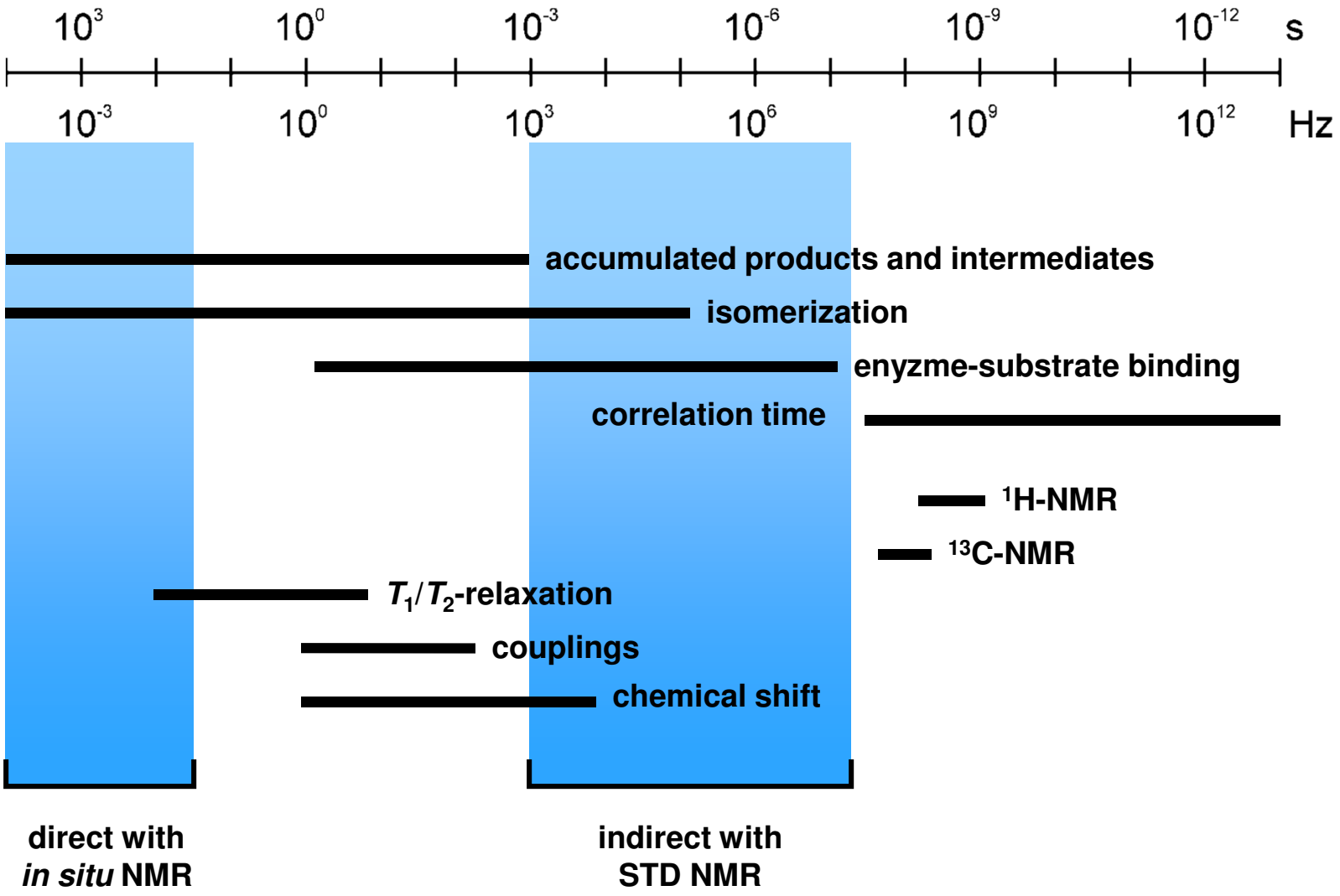
D. W. Ribbons, 1997

Which details of reactions can be investigated by NMR?

Which concepts und methods have to be applied?



Time-Dependence of NMR-Measuring Methods



In situ ^{1}H-NMR Monitoring

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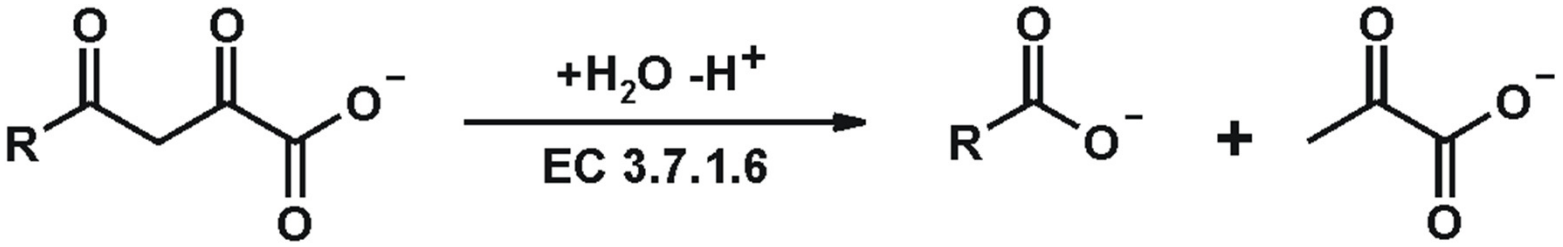
- No sampling necessary
- Reaction is not influenced
- Direct substrate and product analysis possible
- ‘Separation’ can be made in the spectrum
- (Almost) all organic compounds contain protons
- Expensive isotope labeling is not necessary
- Concentrations down to ca. 100 µM are well detectable

- Water-suppression
- D_2O must be added for a Lock-signal
- Small spectral width

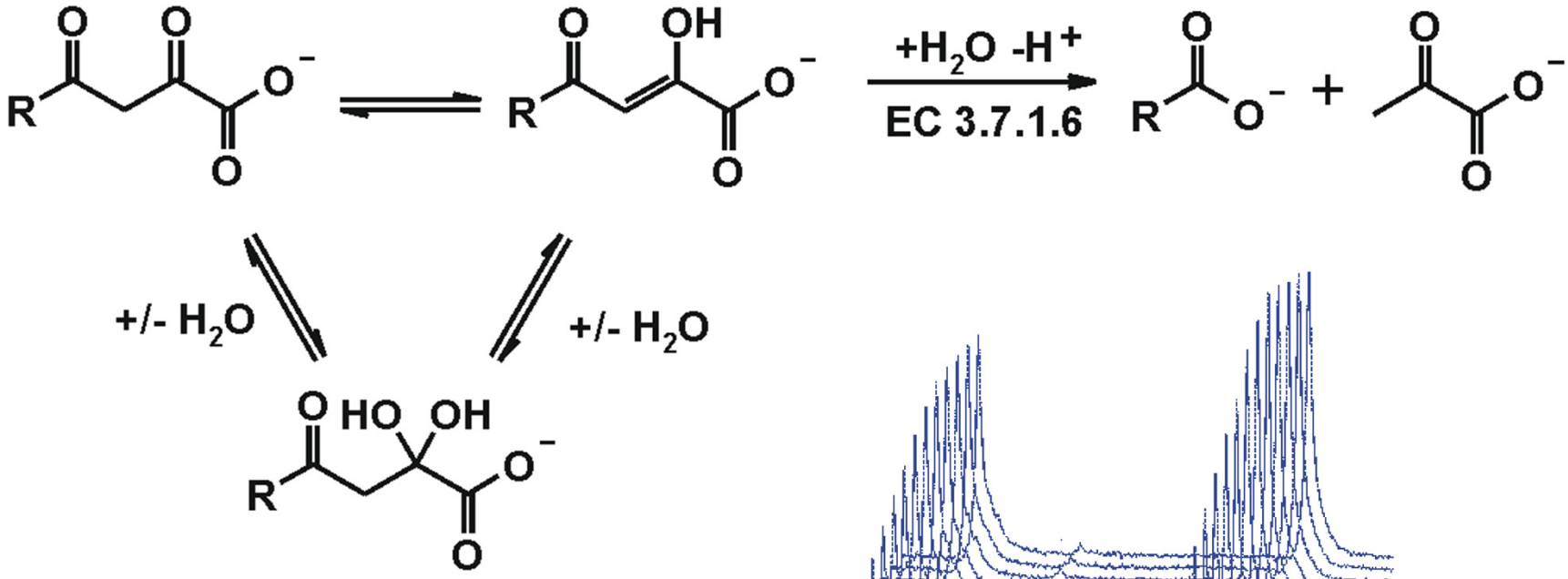
Brecker, Ribbons. *Trends Biotechnol.* **2000**, *18*, 197.
Weber, Brecker. *Curr. Opinions Biotechnol.* **2000**, *11*, 572.

β-Ketolase – Reaction

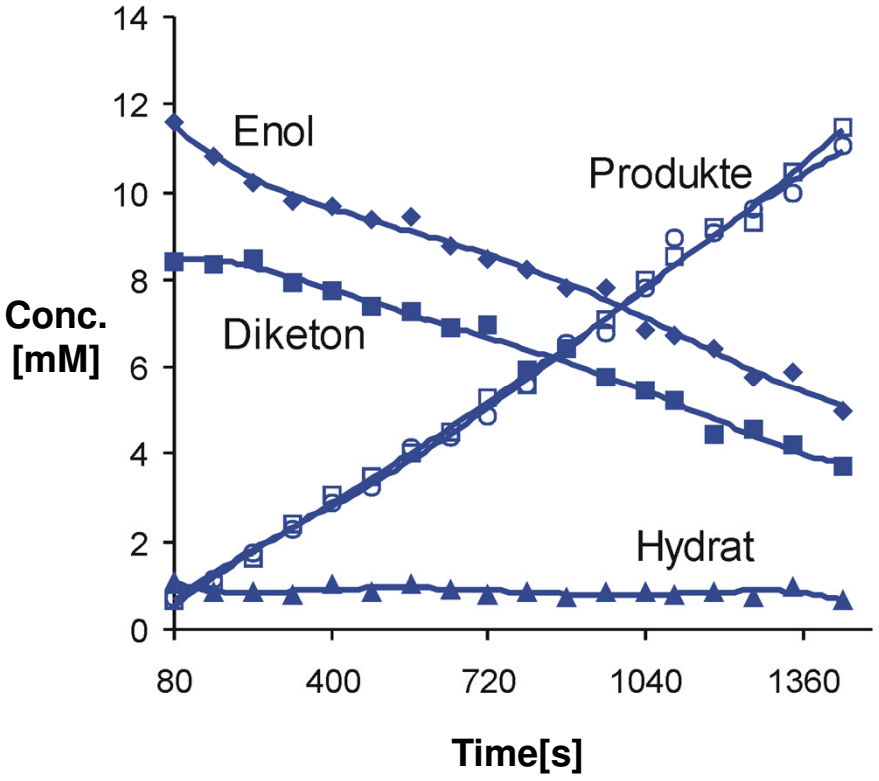
Isolated from *Pseudomonas putida* ORC

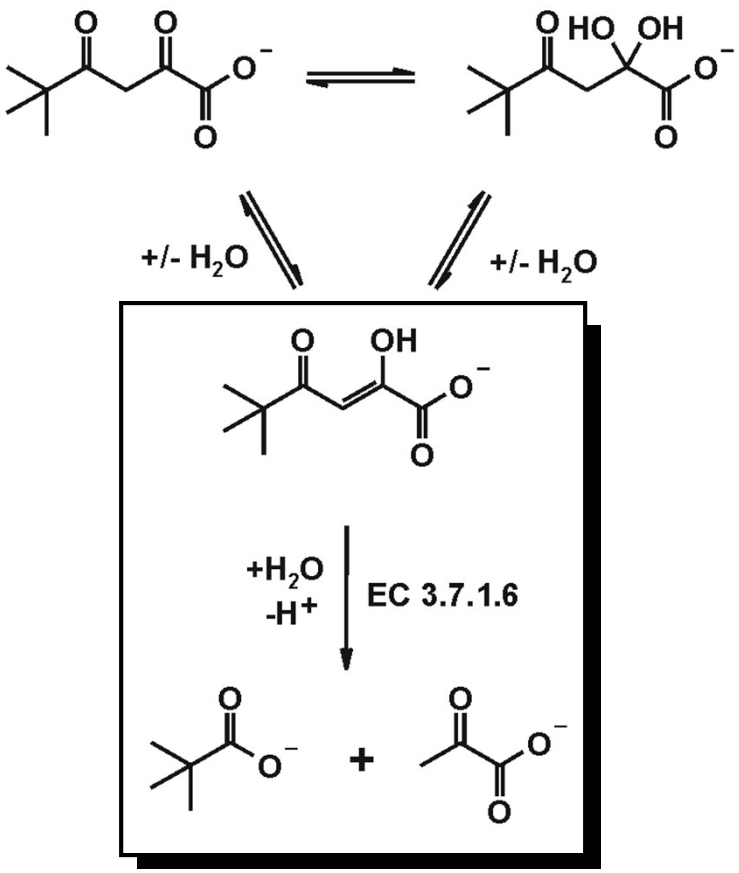


β-Ketolase – Enzyme Catalysed Hydrolysis



β-Ketolase – Hydrolysis of the Enol-Form

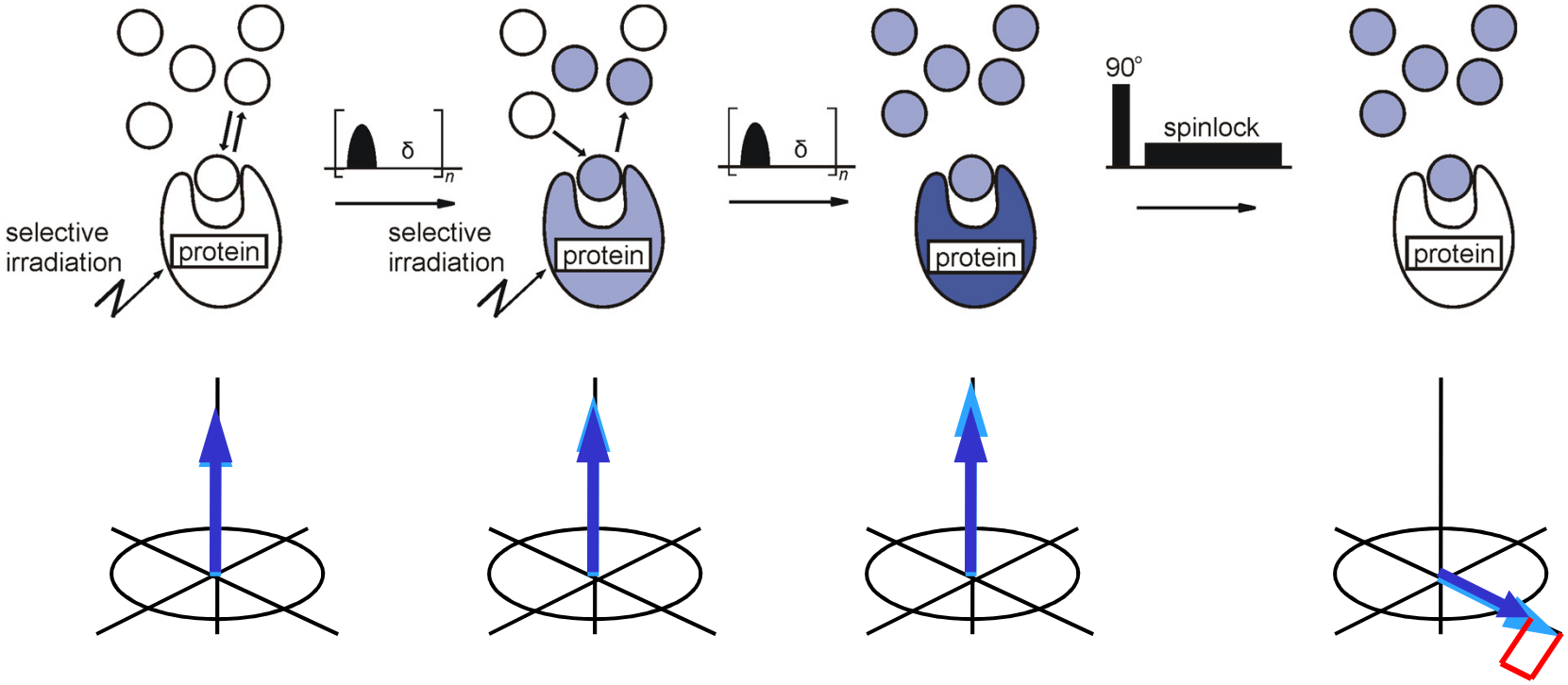




Brecker et al. *New J. Chem.* **1999**, *23*, 437.
Pokorny et al. *J. Bacteriol.* **1999**, *181*, 5051.

Saturation Transfer Difference (STD) NMR

Principle



1. Experiment: Selective irradiation of a protein frequency
2. Experiment: Irradiation at ~40 ppm

Saturation Transfer Difference (STD) NMR

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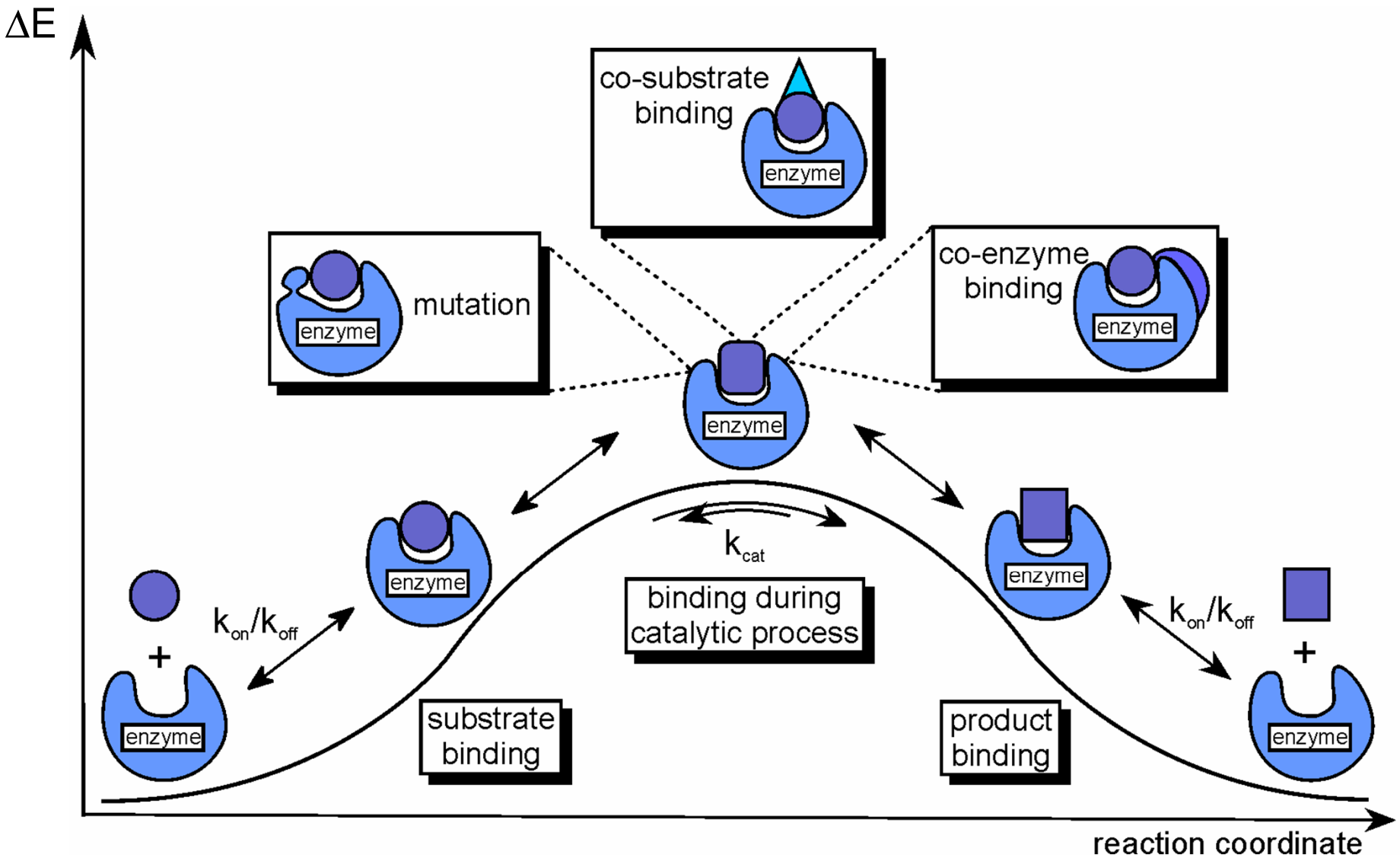
Premises

- Small amounts of the protein [µg scale]
- Isotopic labeling is not necessary
- Independent of knowledge about the protein structure

Results

- Spatial closeness of ligand-protons to the protein
- Binding epitope of the ligands
- Information about protein-ligand interactions

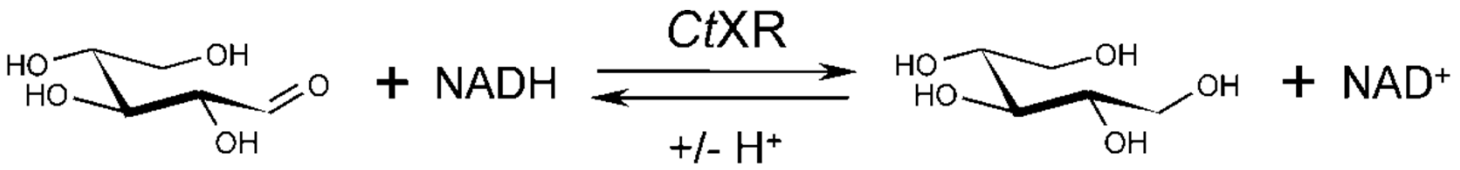
STD NMR in Biocatalysis



Brecker et al. *Carbohydr. Res.* **2008**, *343*, 2153.

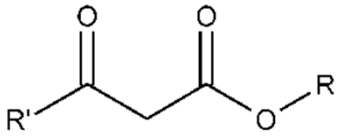
Binding of Intermediates during Reaction

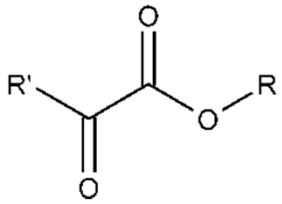
Candida tenuis Xylose Reductase (*Ct*XR)

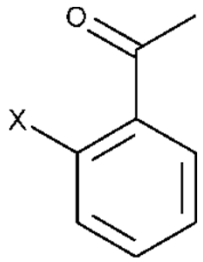


Acceptance of a very broad variety of unnatural substrates

- α-keto-ester
- β-keto-ester
- *ortho*-halo-acetophenone







Binding of Intermediates during Reaction

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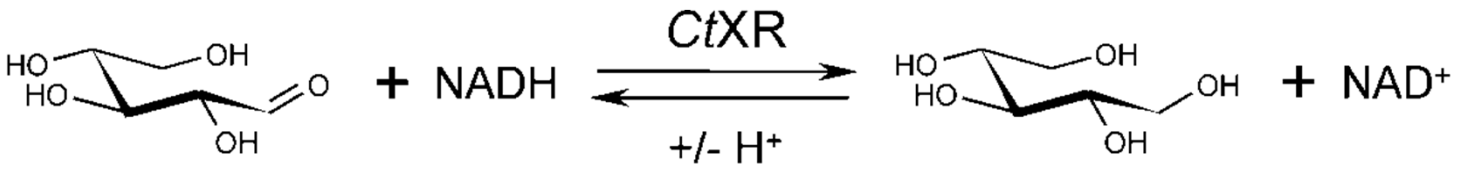
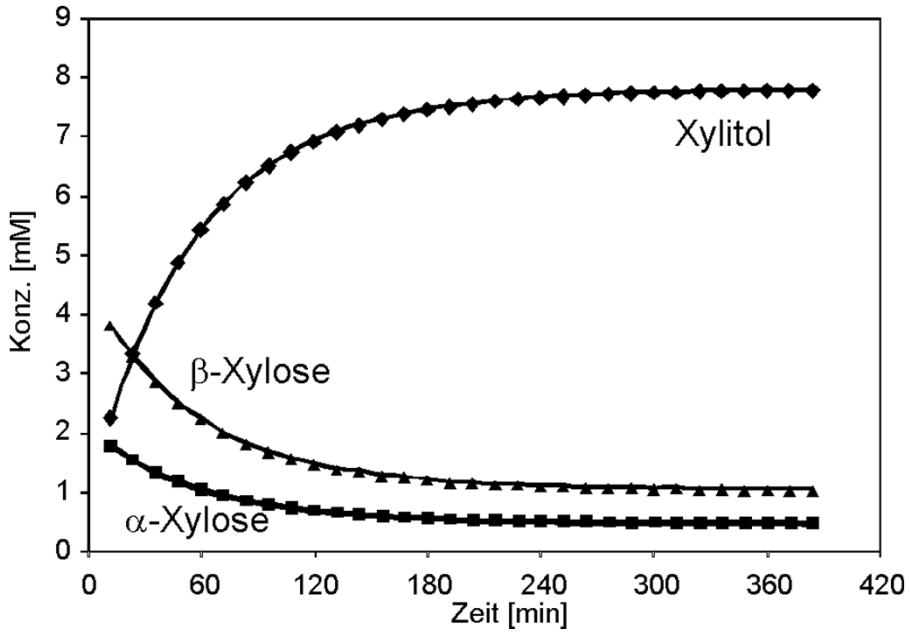


Very high catalytic efficiency: 680,000 M^{-1} s^{-1}

In situ NMR of *Ct*XR catalysed xylose reduction at 303 K



In situ NMR of *Ct*XR catalysed reductions

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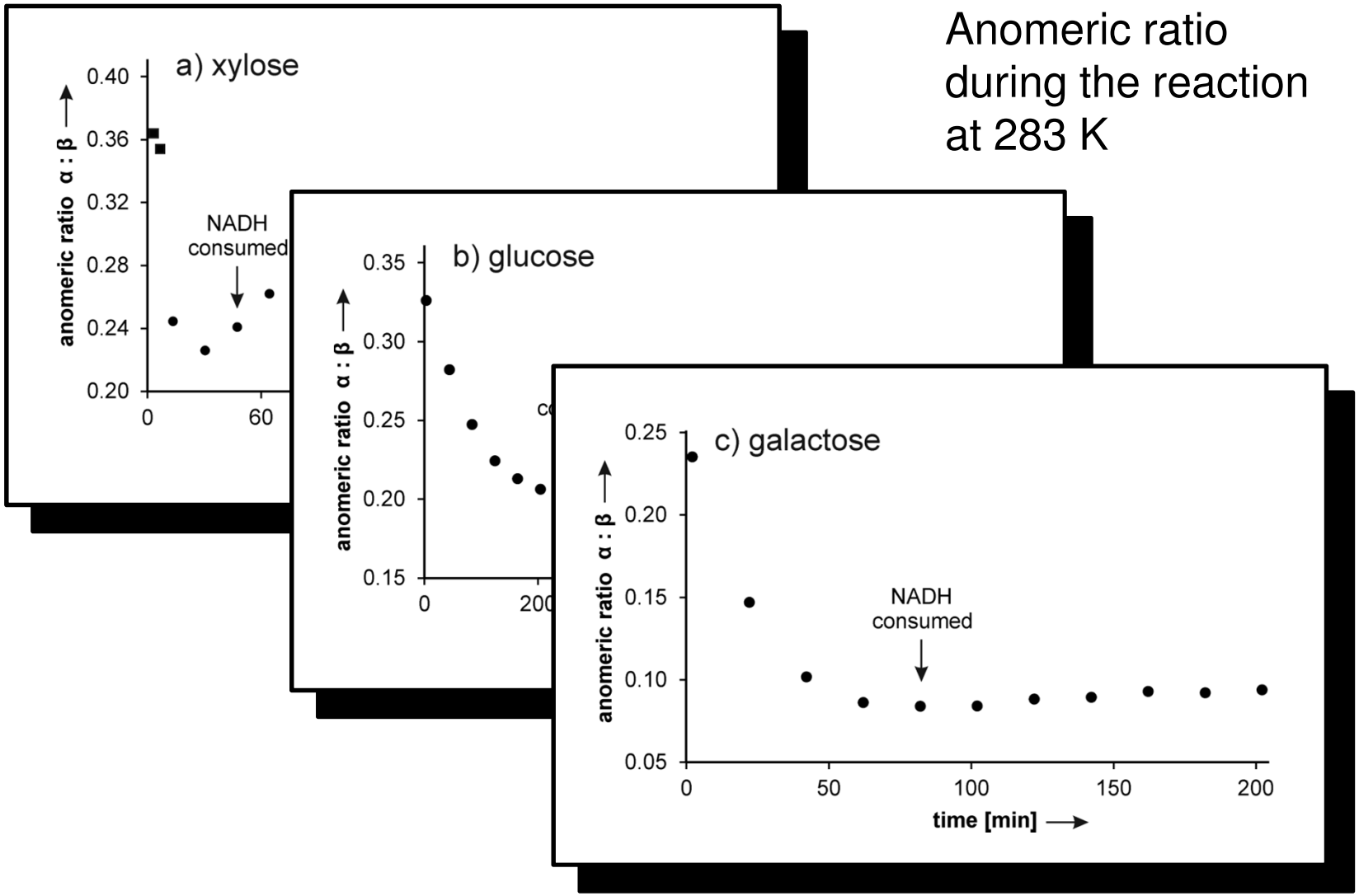
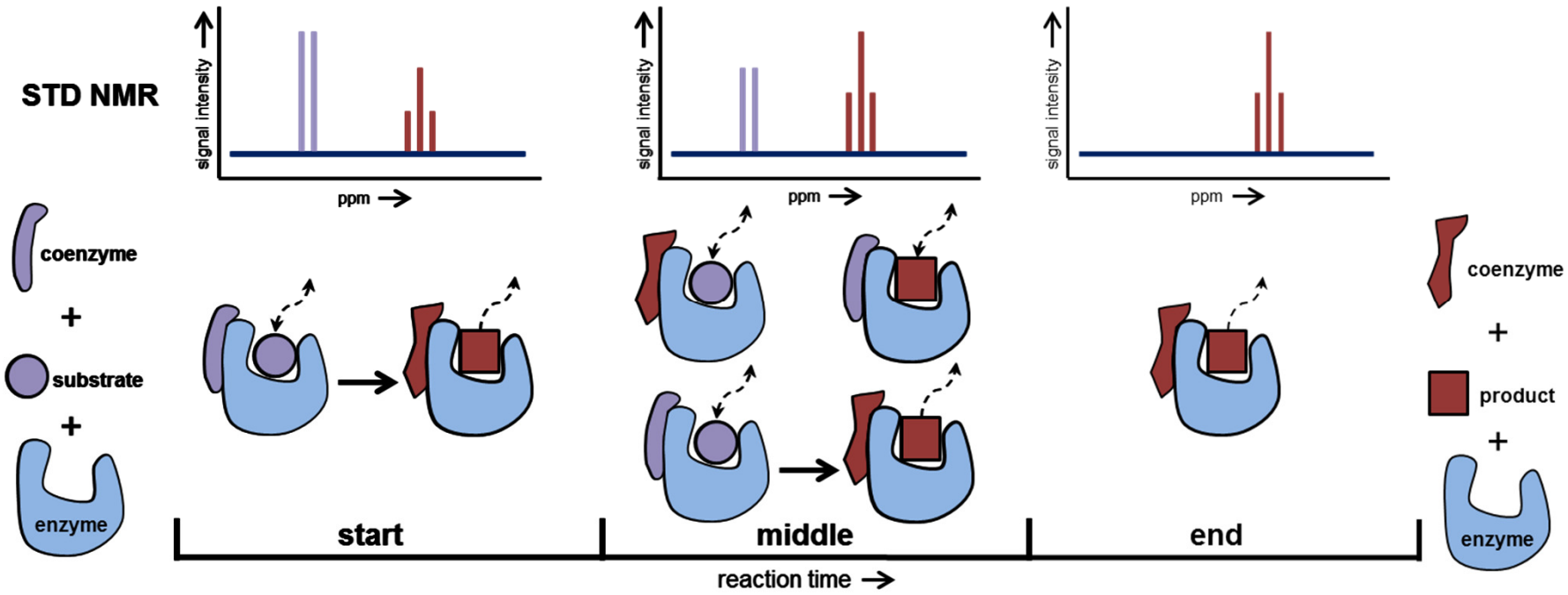
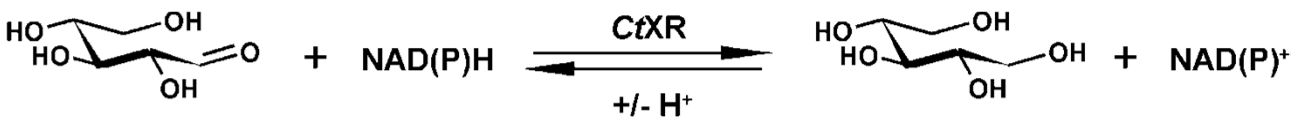


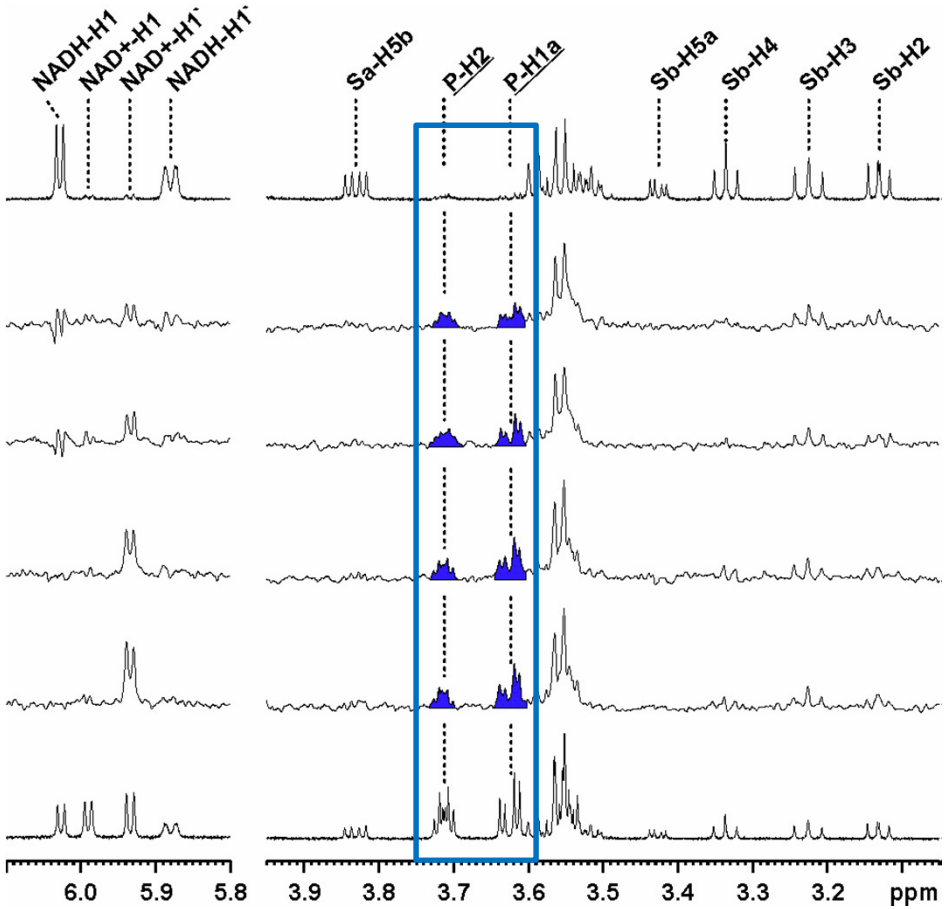
Anomeric ratio during the reaction at 283 K

STD NMR of Ternary Complexes



Binding of Intermediates during Reaction





^{1}H-NMR 0 min

STD-NMR 1-15 min

STD-NMR 16-30 min

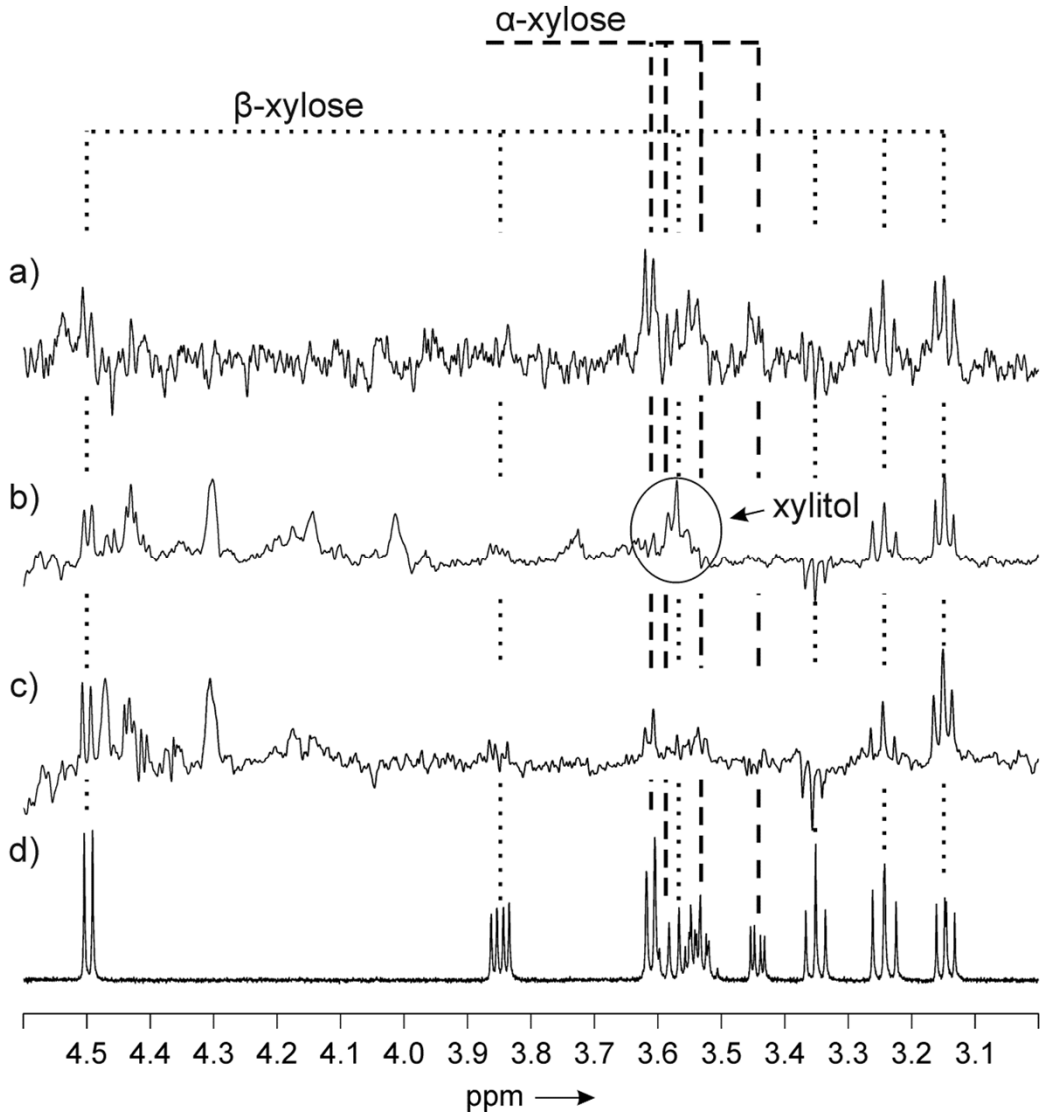
STD-NMR 31-45 min

STD-NMR 46-60 min

^{1}H-NMR 61 min

Brecker et al. *Carbohydr. Res.* **2008**, *343*, 2153.

STD NMR of Xyl/Coenzym/*Ct*XR-Complexes



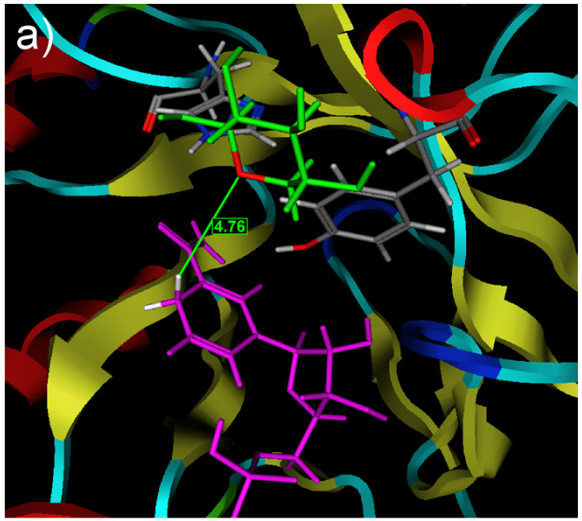
STD NMR Xyl/*Ct*XR

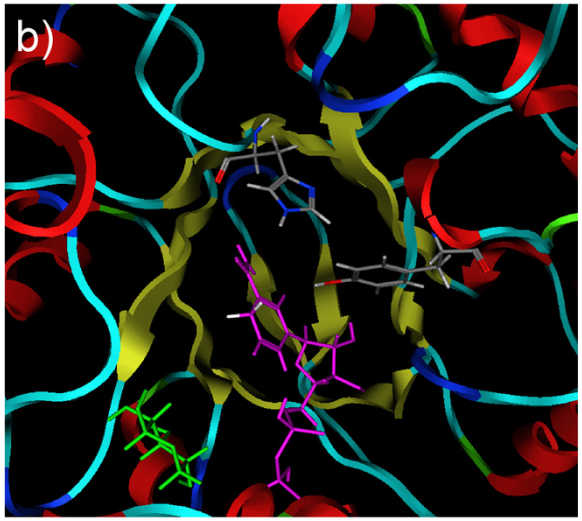
STD NMR Xyl/NADH/*Ct*XR

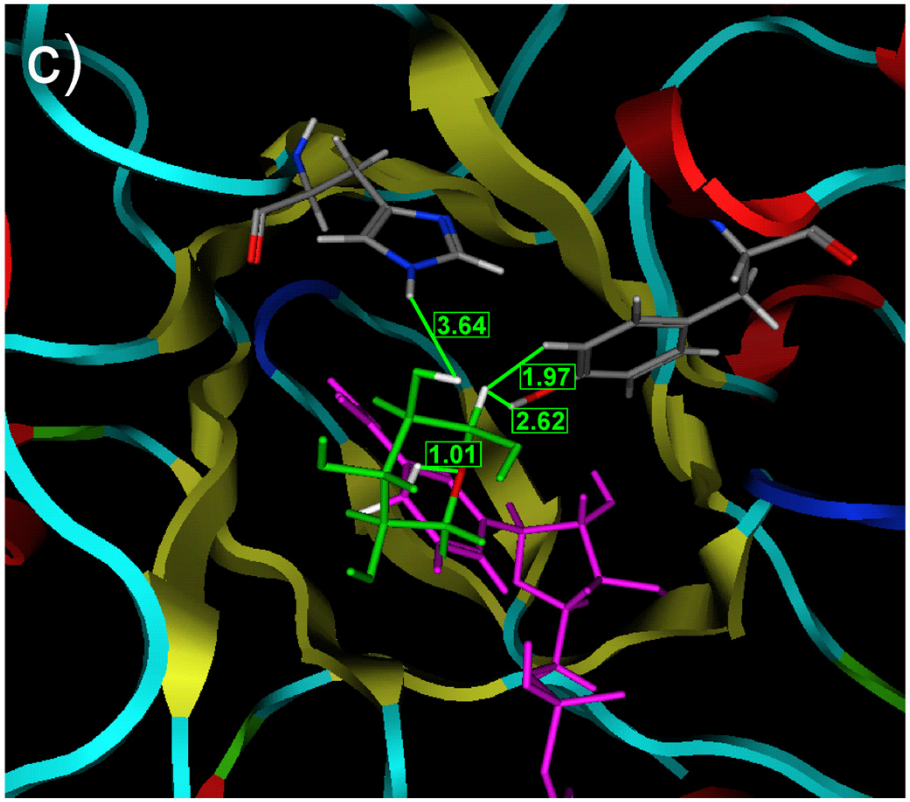
STD NMR Xyl/NAD^+/*Ct*XR

^{1}H NMR Xyl

Molecular Docking in the Active Site







Vogl, Brecker. *RSC Adv.* **2013**, *3*, 25997.

Candida tenuis Xylose Reductase (*Ct*XR)

Acceptance of a very broad variety of unnatural substrates

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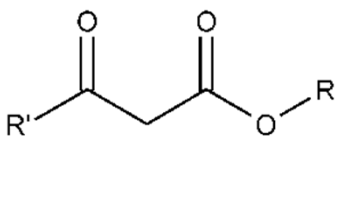
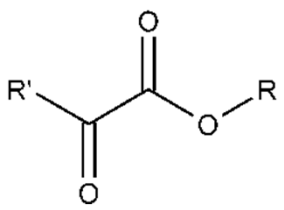
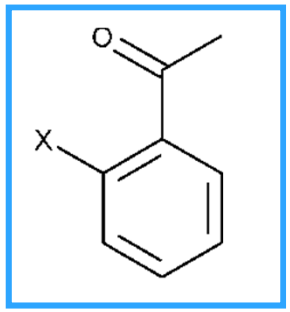
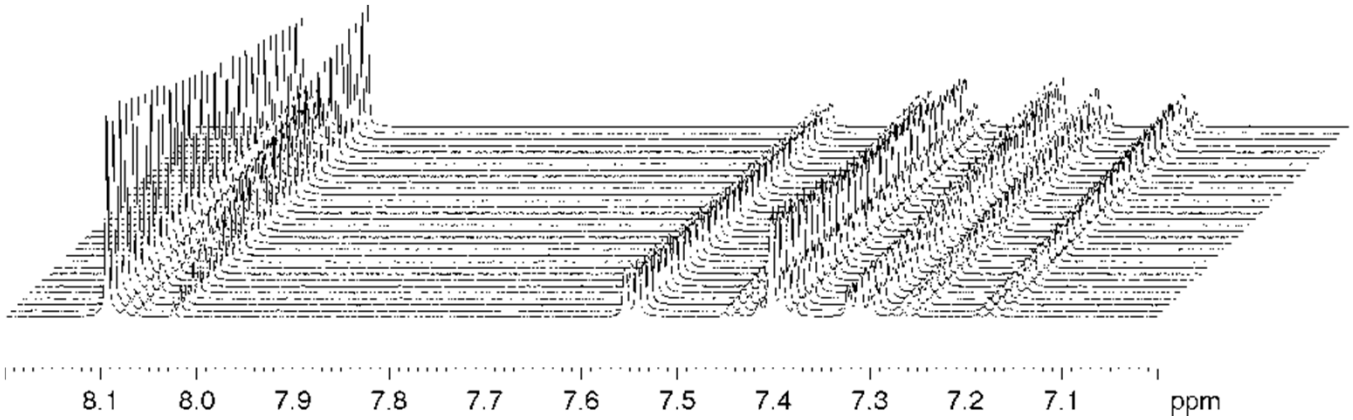
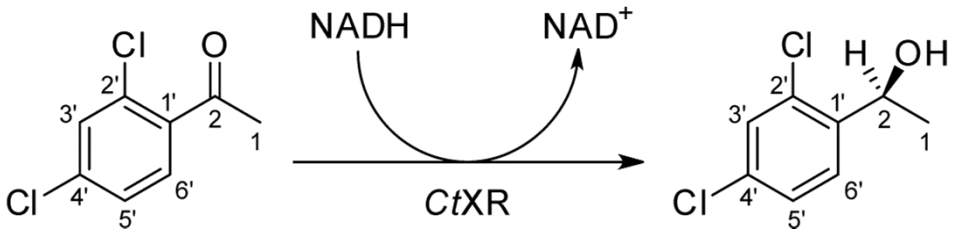


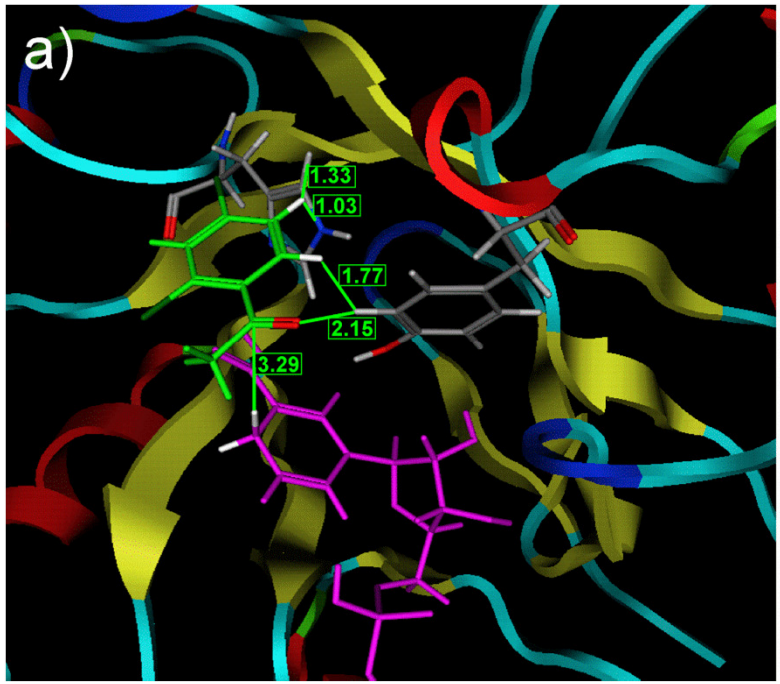


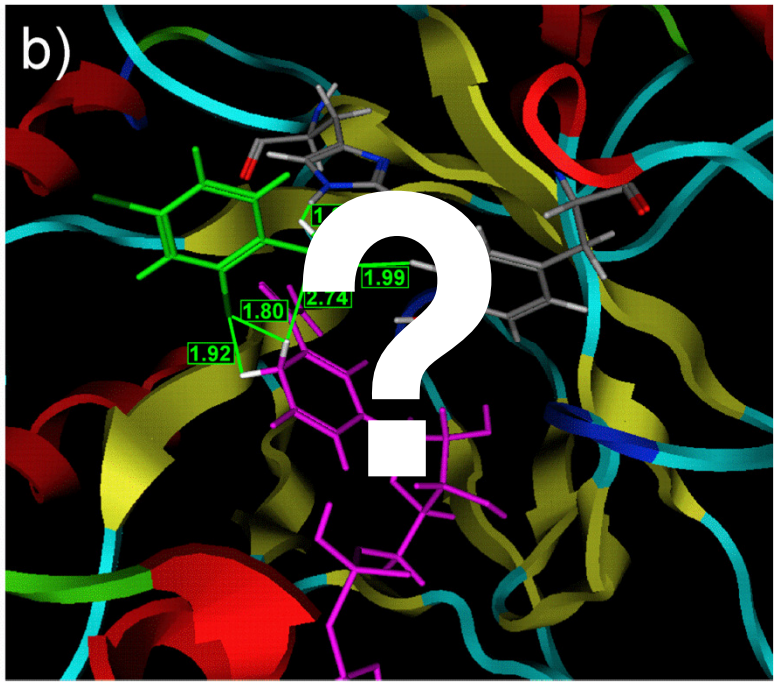




Molekular Docking of an Acetophenone



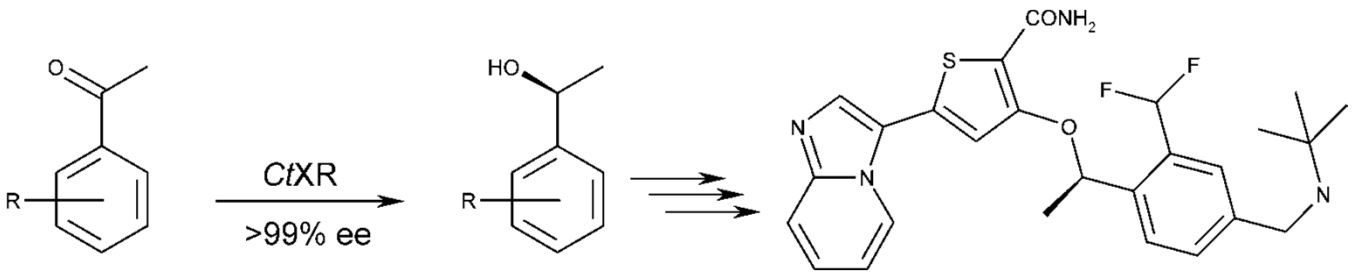




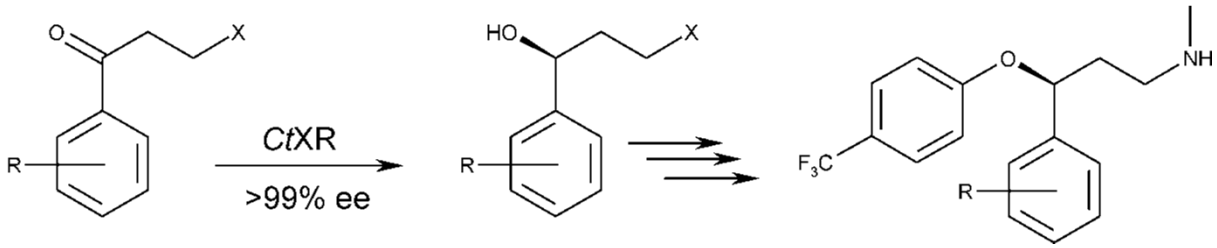
Vogl, Brecker. *RSC Adv.* **2013**, *3*, 25997.

Applications in Synthesis of Drugs

2-Chlor-acetophenone as substrate for PLK-1 inhibitor (cancer therapy)



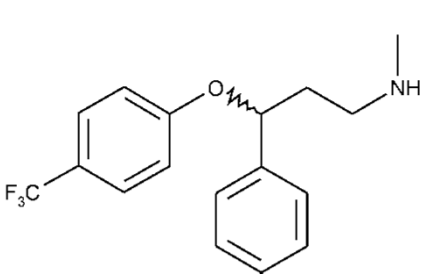
Propiophenone as substrate for oxetines (antidepressent drug)



Kratzer et al. *Biotechnol. Bioeng.* **2011**, *108*, 797.
Vogl et al. *Org. Biomol. Chem.* **2011**, *9*, 5863.
PLK-1 Hemmer: Sato et al. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4673.

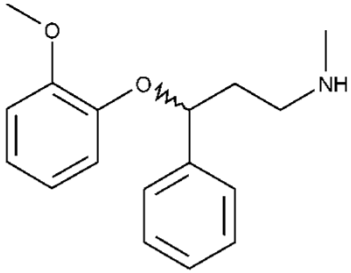
Oxetines (Antidepressent Drugs)

Fluoxetin

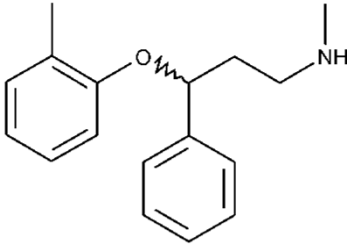


„Prozac“

Nisoxetin



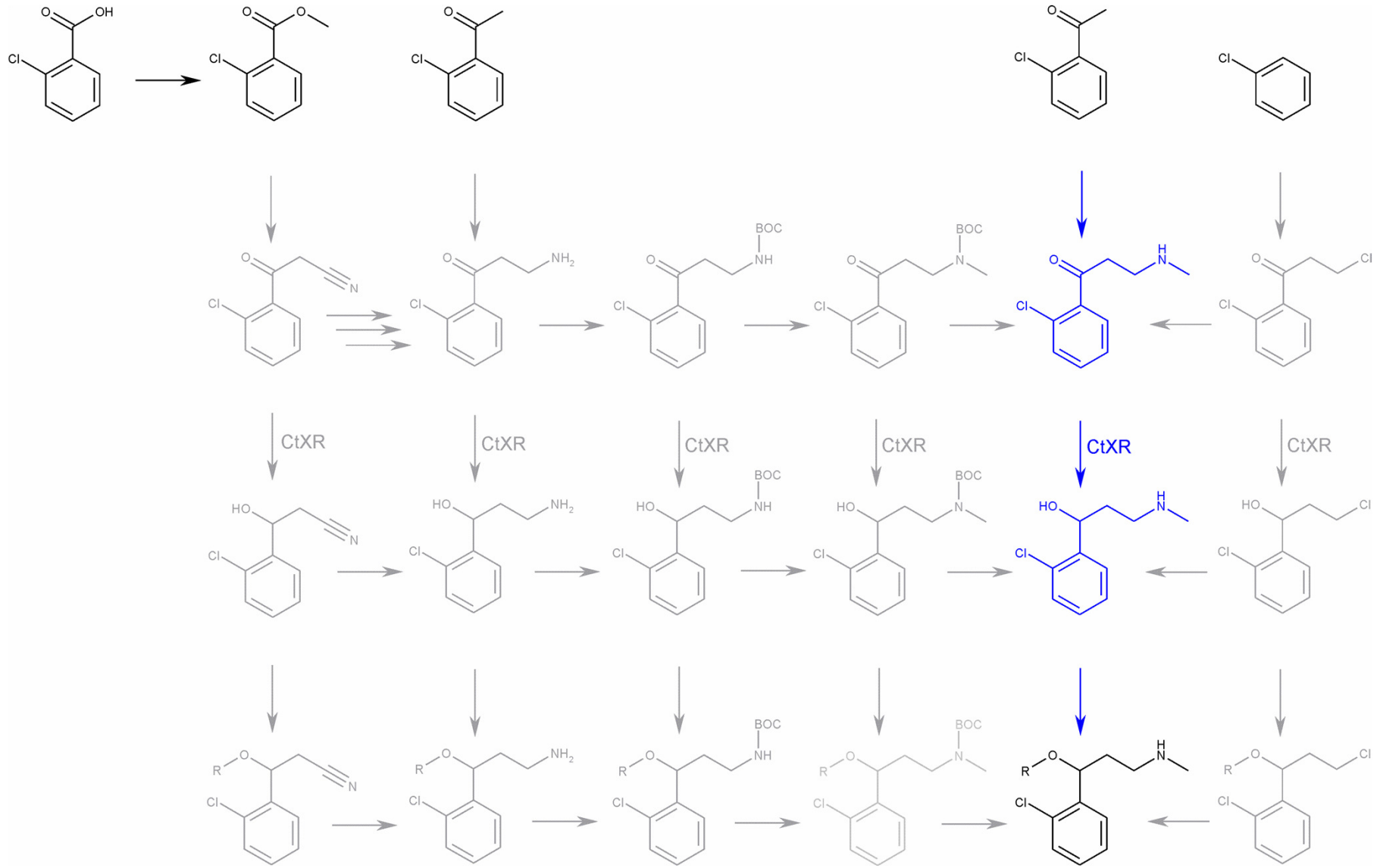
Tomoxetin



- Worldwide use as antidepressent drug
- Sales volume in the billion-dollar region
- Application as racemic mixtures
- Side effects lead to intensive critique

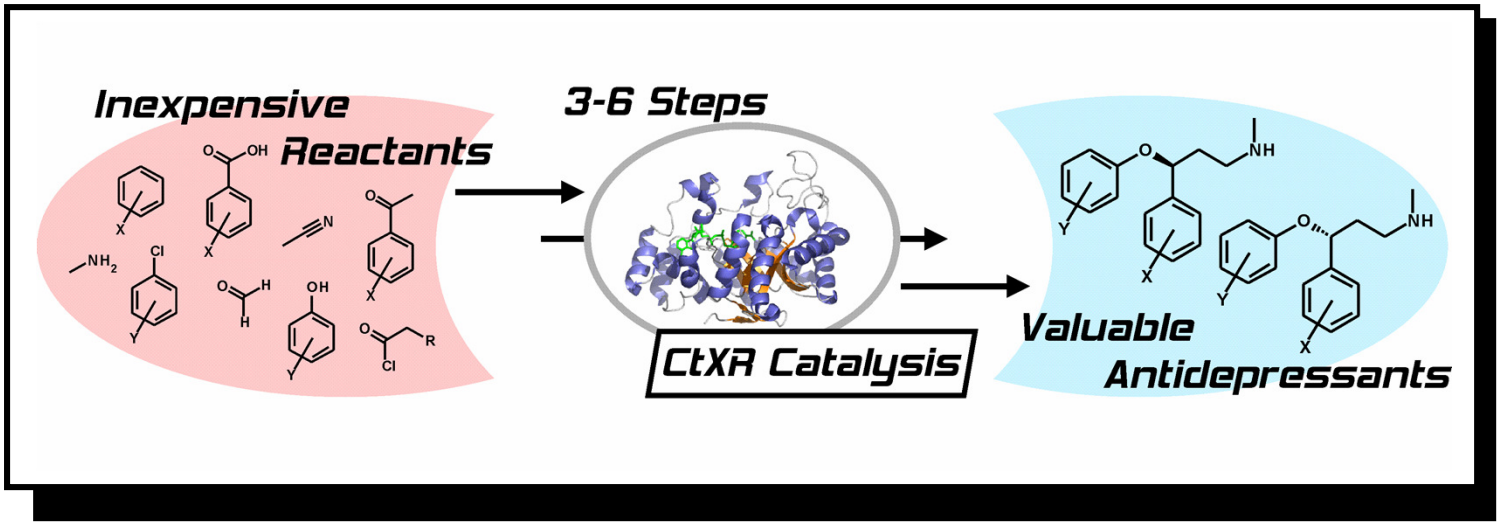
→ Enantiomerical pure synthesis using *Ct*XR

Enantiomerical pure synthesis using *Ct*XR



Patent application

Patent application and marketing since 2010



Vogl et al. *Chirality* **2012**, *24*, 847; Brecker et al. EP 2348120 B1, **2014**, 28 pp.

Acknowledegent

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