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Biocatalytic Synthesis

Enzymatic solutions for chemical problems

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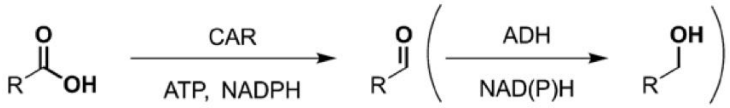
Reductive Biotransformation

Reduction of Carboxylic Acids



CAR = carboxylate reductase; ADH = alcohol dehydrogenase

K. Napora-Wijata, K. Robins, A. Osorio-Lozada, et al., Whole-Cell Carboxylate Reduction for the Synthesis of 3-Hydroxytyrosol. *ChemCatChem.* **6**, 1089–1095 (2014).

D. Schwendenwein, G. Fiume, H. Weber, et al., Selective Enzymatic Transformation to Aldehydes *in vivo* by Fungal Carboxylate Reductase from *Neurospora crassa*. *Adv. Synth. Catal.* **358**, 3414–3421 (2016).

M. Winkler, Carboxylic acid reductase enzymes (CARs). *Curr. Opin. Chem. Biol.* **43**, 23–29 (2018).

Reduction of Aldehydes and Ketones

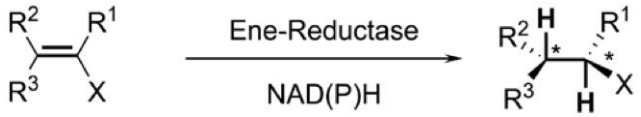
ADH = alcohol dehydrogenase

W. Stampfer, B. Kosjek, C. Moitzi, et al., Biocatalytic asymmetric hydrogen transfer. *Angew. Chem. Int. Ed.* **41**, 1014–1017 (2002).

C. V. Voss, C. C. Gruber, W. Kroutil, Deracemization of secondary alcohols through a concurrent tandem biocatalytic oxidation and reduction. *Angew. Chem. Int. Ed.* **47**, 714–745 (2008).

K. Napora, T. M. Wrodnigg, P. Kosmus, et al., *Yarrowia lipolytica* dehydrogenase/reductase: an enzyme tolerant for lipophilic compounds and carbohydrate substrates. *Bioorg. Med. Chem. Lett.* **23**, 3393–3395 (2013).

Reduction of C=C-Bonds



X = electron-withdrawing group

R. Stuermer, B. Hauer, M. Hall, et al., Asymmetric bioreduction of activated C=C bonds using enoate reductases from the old yellow enzyme family. *Curr. Opin. Chem. Biol.* **11**, 203–13 (2007).

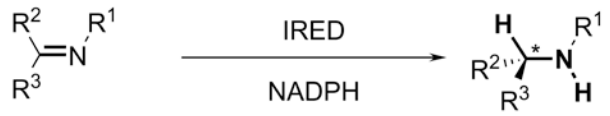
C. K. Winkler, G. Tasnádi, D. Clay, et al., Asymmetric bioreduction of activated alkenes to industrially relevant optically active compounds. *J. Biotechnol.* **162**, 381–389 (2012).

G. Steinkellner, C. C. Gruber, T. Pavkov-Keller, et al., Identification of promiscuous ene-reductase activity by mining structural databases using active site constellations. *Nat. Commun.* **5**, 4150 (2014).

C. K. Winkler, K. Faber, M. Hall, Biocatalytic reduction of activated C=C -bonds and beyond: emerging trends. *Curr. Opin. Chem. Biol.* **43**, 97–105 (2018).

Reductive Biotransformation

Reduction of Imines

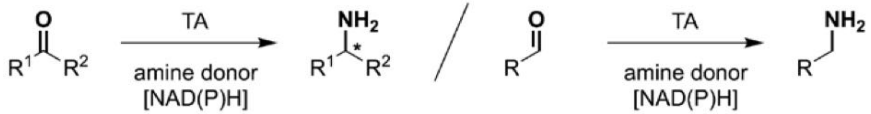


IRED = imine reductase

J. H. Schrittwieser, S. Velikogne, W. Kroutil, Biocatalytic Imine Reduction and Reductive Amination of Ketones. *Adv. Synth. Catal.* **357**, 1655–1685 (2015).

S. Velikogne, V. Resch, C. Dertnig, et al., Sequence-Based *In-silico* Discovery, Characterisation, and Biocatalytic Application of a Set of Imine Reductases. *ChemCatChem.* **10**, 3236–3246 (2018).

Reductive Amination of Aldehydes and Ketones



TA = ω-transaminase

D. Koszelewski, I. Lavandera, D. Clay, et al., Formal asymmetric biocatalytic reductive amination. *Angew. Chem. Int. Ed.* **47**, 9337–9340 (2008).

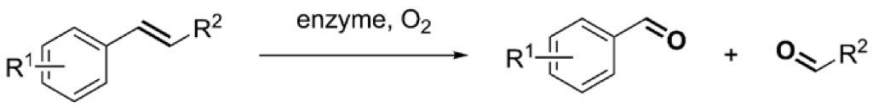
W. Kroutil, E. M. Fischereder, C. S. Fuchs, et al., Asymmetric preparation of prim -, sec -, and tert-amines employing selected biocatalysts. *Org. Process Res. Dev.* **17**, 751–759 (2013).

R. C. Simon, N. Richter, E. Busto, et al., Recent developments of cascade reactions involving ω-transaminases. *ACS Catal.* **4**, 129–143 (2014).

R. C. Simon, B. Grischek, F. Zepeck, et al., Regio- and stereoselective monoamination of diketones without protecting groups. *Angew. Chem. Int. Ed.* **51**, 6713–6716 (2012).

Oxidative Biotransformation

C=C-Bond Cleavage



H. Mang, J. Gross, M. Lara, et al., Biocatalytic single-step alkene cleavage from aryl alkenes: An enzymatic equivalent to reductive ozonization. *Angew. Chem. Int. Ed.* **45**, 5201–5203 (2006).

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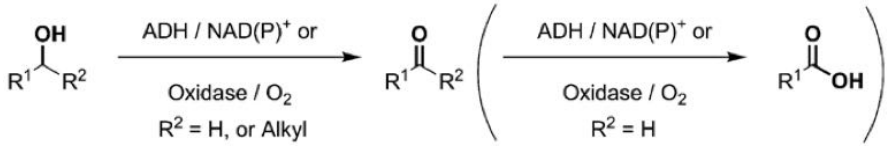
C. E. Paul, A. Rajagopalan, I. Lavandera, et al., Expanding the regioselective enzymatic repertoire: Oxidative mono-cleavage of dialkenes catalyzed by Trametes hirsuta. *Chem. Commun.* **48**, 3303 (2012).

A. Rajagopalan, M. Lara, W. Kroutil, Oxidative alkene cleavage by chemical and enzymatic methods. *Adv. Synth. Catal.* **355,** 3321–3335 (2013).

Oxidation of Alcohols / Aldehydes



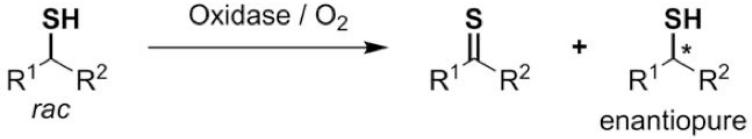
ADH = alcohol dehydrogenase

W. Stampfer, B. Kosjek, C. Moitzi, et al., Biocatalytic asymmetric hydrogen transfer. *Angew. Chem. Int. Ed.* **41**, 1014–1017 (2002).

C. V. Voss, C. C. Gruber, W. Kroutil, Deracemization of secondary alcohols through a concurrent tandem biocatalytic oxidation and reduction. *Angew. Chem. Int. Ed.* **47**, 714–745 (2008).

C. Wuensch, H. Lechner, S. M. Glueck, et al., Asymmetric biocatalytic cannizzaro-type reaction. *ChemCatChem.* **5**, 1744–1748 (2013).

Oxidation of Thiols

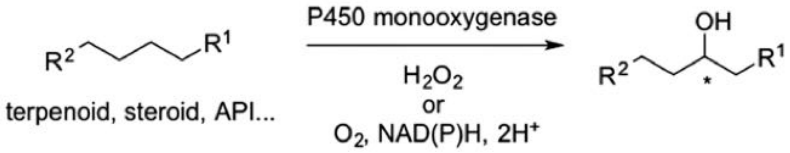


TA = transaminase; AO = amine oxidase

M. Pickl, A. Swoboda, E. Romero, et al., Kinetic Resolution of sec-Thiols by Enantioselective Oxidation with Rationally Engineered 5-(Hydroxymethyl) furfural Oxidase. *Angew. Chem. Int. Ed.* **57**, 2864–2868 (2018)

Oxidative Biotransformation

Enzymatic Hydroxylation



A. Glieder, E. T. Farinas, F. H. Arnold, Laboratory evolution of a soluble, self-sufficient, highly active alkane hydroxylase. *Nat. Biotechnol.* **20(11)**, 1135–1139 (2002).

A. K. Migglautsch, M. Willim, B. Schweda, et al., Aliphatic hydroxylation and epoxidation of capsaicin by cytochrome P450 CYP505X. *Tetrahedron.* **74(43)**, 6199–6204 (2018).

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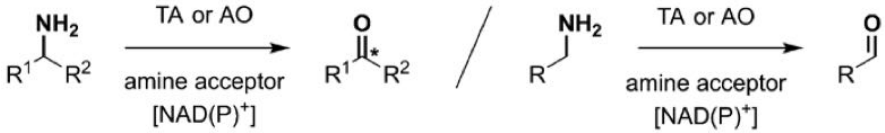
T. Wriessnegger, S. Moser, A. Emmerstorfer-Augustin, et al., Enhancing cytochrome P450-mediated conversions in *P. pastoris* through RAD52 over -expression and optimizing the cultivation conditions. *Fungal Genet. Biol.* **89**, 114–125 (2016).

A. Emmerstorfer, M. Wimmer-Teubenbacher, T. Wriessnegger, et al., Over-expression of ICE2 stabilizes cytochrome P450 reductase in Saccharo-myces cerevisiae and *Pichia pastoris. Biotechnol. J.* **10**, 623–635 (2015).

Enzymatic De-Amination



TA = transaminase; AO = amine oxidase

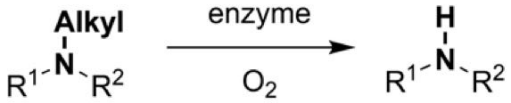
D. Koszelewski, I. Lavandera, D. Clay, et al., Formal asymmetric biocatalytic reductive amination. Angew. Chem. Int. Ed. **47(48)**, 9337–9340 (2008).

W. Kroutil, E. M. Fischereder, C. S. Fuchs, et al., Asymmetric preparation of prim -, sec -, and tert-amines employing selected biocatalysts. *Org. Process Res. Dev.* **17(5)**, 751–759 (2013).

R. C. Simon, N. Richter, E. Busto, et al., Recent developments of cascade reactions involving ω-transaminases. *ACS Catal.* **4(1)**, 129–143 (2014).

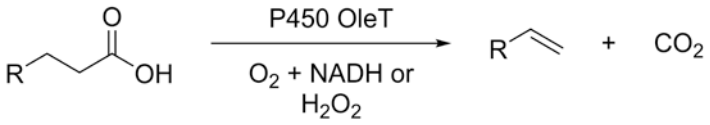
R. C. Simon, B. Grischek, F. Zepeck, et al., Regio- and stereoselective monoamination of diketones without protecting groups. *Angew. Chem. Int. Ed.* **51(27)**, 6713–6716 (2012).

Enzymatic De-Alkylation



S. Gandomkar, E. M. Fischereder, J. H. Schrittwieser, et al., Enantioselective Oxidative Aerobic Dealkylation of N-Ethyl Benzylisoquinolines by Employing the Berberine Bridge Enzyme. *Angew. Chem. Int. Ed.* **54(50)**, 15051–15054 (2015).

Oxidative Decarboxylation

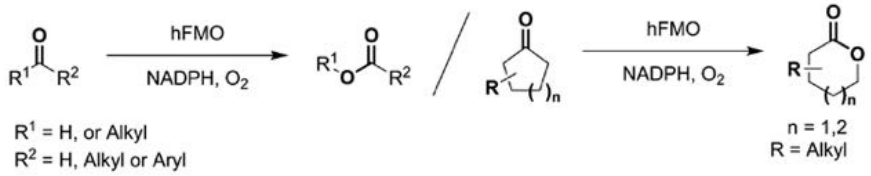


A. Dennig, M. Kuhn, S. Tassoti, et al., Oxidative Decarboxylation of Short-Chain Fatty Acids to 1-Alkenes. *Angew. Chem. Int. Ed.* **54(30)**, 8819–8822 (2015).

I. Zachos, S. K. Gassmeyer, D. Bauer, et al., Photobiocatalytic decarboxylation for olefin synthesis. *Chem. Commun. (Camb).* **51**, 1918–21 (2015).

A. Dennig, S. Kurakin, M. Kuhn, et al., Enzymatic Oxidative Tandem Decarboxylation of Dioic Acids to Terminal Dienes. *European J. Org. Chem.* **21**, 3473–3477 (2016).

Baeyer-Villiger Oxidation



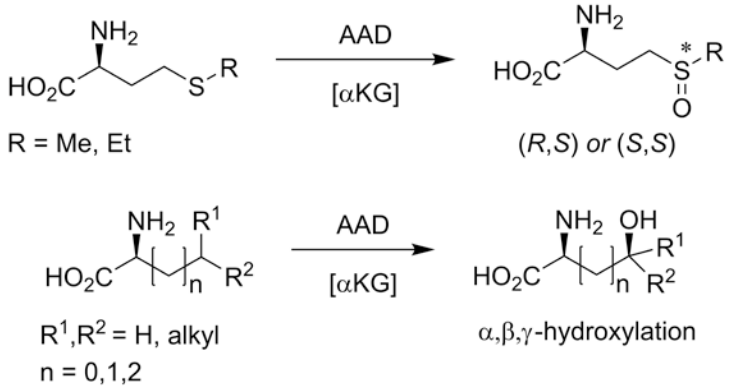
hFMO = human flavin containing monooxygenase

F. Fiorentini, M. Geier, C. Binda, et al., Biocatalytic Characterization of Human FMO5: Unearthing Baeyer-Villiger Reactions in Humans. *ACS Chem. Biol.* **11(4)**, 1039–1048 (2016).

F. Fiorentini, E. Romero, M. W. Fraaije, et al., Baeyer-Villiger Monooxygenase FMO5 as Entry Point in Drug Metabolism. *ACS Chem. Biol.* **12(9)**, 2379–2387 (2017).

Oxidative Biotransformation

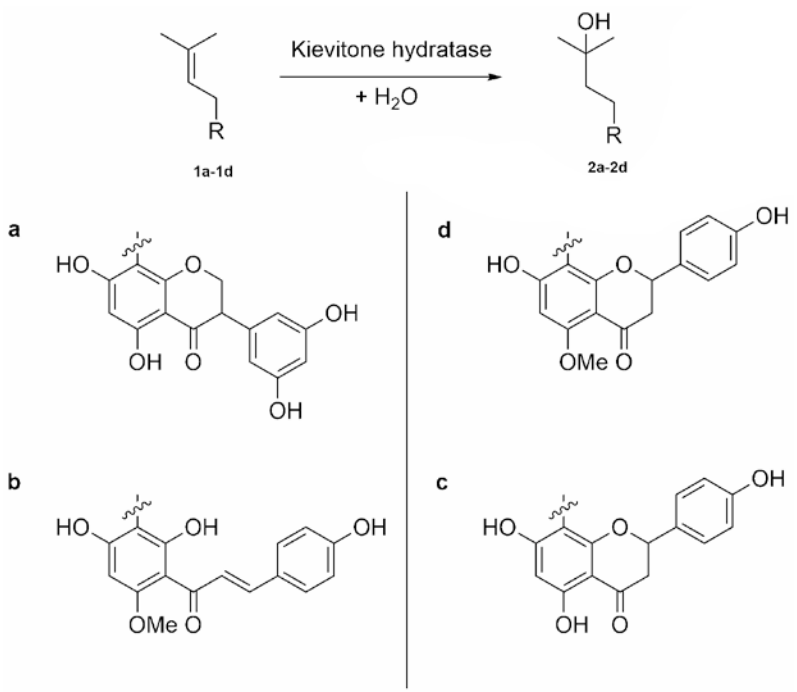
Oxyfunctionalisation of Amino Acids



aKG = α-ketoglutarate; AAD = amino acid dioxygenase;

J. Enoki, J. Meisborn, A. C. Müller, et al., A multi-enzymatic cascade reaction for the stereo-selective production of γ-oxyfunctionalyzed amino acids. *Front. Microbiol.* **7**, 425 (2016).

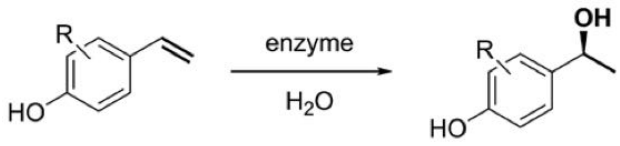
Selective Hydration of Flavonoids



M. Engleder, M. Horvat, A. Emmerstorfer-Augustin, et al., Recombinant expression, purification and biochemical characterization of kievitone hydratase from *Nectria haematococca*. *PLoS One.* **13**, e0192653 (2018).

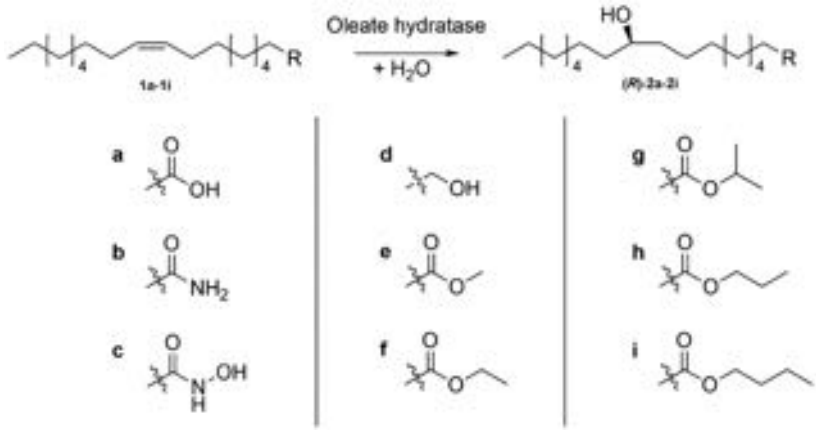
Enzymatic Hydration

Hydration of Hydroxystyrene Derivatives



C. Wuensch, J. Gross, G. Steinkellner, et al., Asymmetric Enzymatic Hydration of Hydroxystyrene Derivatives. *Angew. Chemie Int. Ed.* **52**, 2293–2297 (2013).

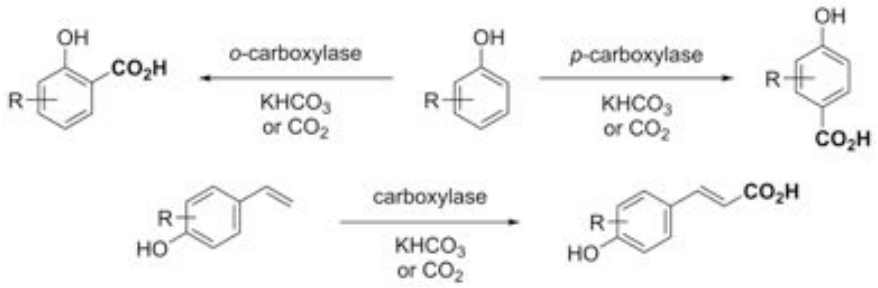
Asymmetric Hydration of Olefins



M. Engleder, G. A. Strohmeier, H. Weber, et al., Evolving the Promiscuity of *Elizabethkingia meningoseptica* Oleate Hydratase for the Regio- and Stereoselective Hydration of Oleic Acid Derivatives. *Angew. Chem. Int. Ed*. (2019), doi:10.1002/anie.201901462.

C-C Bond Formation

Bio-Carboxylation



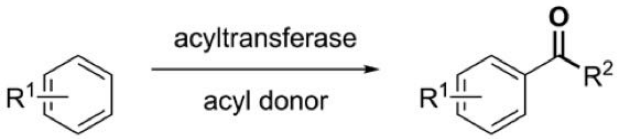
S. M. Glueck, S. Gümüs, W. M. F. Fabian, et al., Biocatalytic carboxylation. *Chem. Soc. Rev.* **39**, 313–328 (2010).

C. Wuensch, S. M. Glueck, J. Gross, et al., Regioselective enzymatic carboxylation of phenols and hydroxystyrene derivatives. *Org. Lett.* **14**, 1974–1977 (2012).

C. Wuensch, N. Schmidt, J. Gross, et al., Pushing the equilibrium of regio-complementary carboxylation of phenols and hydroxystyrene derivatives. *J. Biotechnol.* **168**, 264–270 (2013).

S. E. Payer, S. A. Marshall, N. Bärland, et al., Regioselective para-Carboxylation of Catechols with a Prenylated Flavin Dependent Decarboxylase. *Angew. Chem. Int. Ed.* **56**, 13893–13897 (2017).

Bio-Friedel-Crafts Acylation

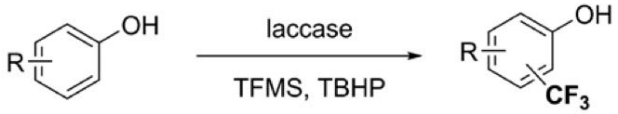


A. Żądło-Dobrowolska, N. G. Schmidt, W. Kroutil, Thioesters as Acyl Donors in Biocatalytic Friedel-Crafts-type Acylation Catalyzed by Acyltransferase from *Pseudomonas protegens*. *ChemCatChem.* **11**, 1064–1068 (2019).

N. G. Schmidt, W. Kroutil, Acyl Donors and Additives for the Biocatalytic Friedel–Crafts Acylation. *European J. Org. Chem.* **39,** 5865–5871 (2017).

N. G. Schmidt, T. Pavkov-Keller, N. Richter, et al., Biocatalytic Friedel-Crafts Acylation and Fries Reaction. *Angew. Chemie Int. Ed.* **56**, 7615–7619 (2017).

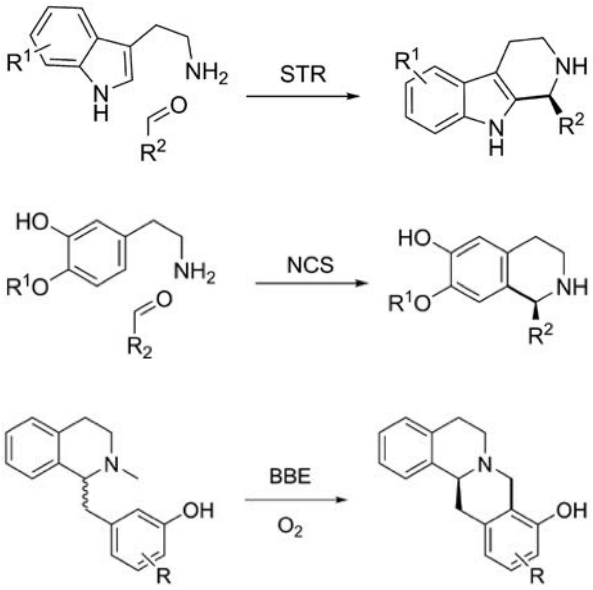
Trifluoromethylation



TFMS = trifluoromethylsulfonic acid; TBHP = tert-butylhydroperoxide

S. E. Payer, S. A. Marshall, N. Bärland, et al., Regioselective para-Carboxylation of Catechols with a Prenylated Flavin Dependent Decarboxylase. *Angew. Chem. Int. Ed.* **56**, 13893–13897 (2017).

Biocatalytic Alkaloid Synthesis



BBE = berberine bridge enzyme; STR = strictosidine synthase; NCS = norcoclaurine synthase

W. Kroutil, E. M. Fischereder, C. S. Fuchs, et al., Asymmetric preparation of prim -, sec -, and tert-amines employing selected biocatalysts. *Org. Process Res. Dev.* **17,** 751–759 (2013).

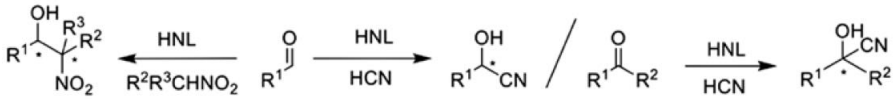
J. H. Schrittwieser, V. Resch, J. H. Sattler, et al., Biocatalytic enantioselective oxidative C-C coupling by aerobic C-H activation. *Angew. Chem. Int. Ed.* **50,** 1068–1071 (2011).

J. H. Schrittwieser, B. Groenendaal, V. Resch, et al., Deracemization by simultaneous bio-oxidative kinetic resolution and stereoinversion. *Angew. Chem. Int. Ed.* **53**, 3731–3734 (2014).

D. Pressnitz, E. M. Fischereder, J. Pletz, et al., Asymmetric Synthesis of (R)-1-Alkyl-Substituted Tetrahydro-ß-carbolines Catalyzed by Strictosidine Synthases. *Angew. Chem. Int. Ed.* **57**, 10683–10687 (2018).

C-C Bond Formation

Cyanohydrin Synthesis / Henry Reaction



HNL = hydroxynitrile lyase

T. Purkarthofer, K. Gruber, M. Gruber-Khadjawi, et al., A biocatalytic Henry reaction - The hydroxynitrile lyase from *Hevea brasiliensis* also catalyzes nitroaldol reactions. *Angew. Chem. Int. Ed.* **45**, 3454–3456 (2006).

M. Gruber-Khadjawi, T. Purkarthofer, W. Skranc, et al., Hydroxynitrile lyase-catalyzed enzymatic nitroaldol (Henry) reaction. *Adv. Synth. Catal.* **349**, 1445–1450 (2007).

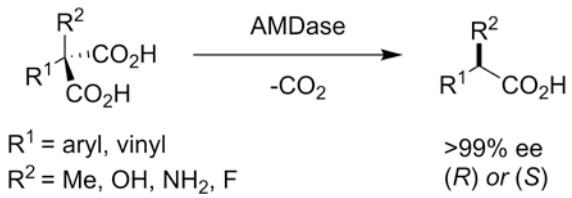
R. Wiedner, B. Kothbauer, T. Pavkov-Keller, et al., Improving the properties of bacterial r-selective hydroxynitrile lyases for industrial applications. *ChemCatChem.* **7**, 325–332 (2015).

A. Glieder, R. Weis, W. Skranc, et al., Comprehensive Step-by-Step Engineering of an (R)-Hydroxynitrile Lyase for Large-Scale Asymmetric Synthesis. *Angew. Chem. Int. Ed.* **42**, 4815–4818 (2003).

E. Lanfranchi, E.-M. Köhler, B. Darnhofer, et al., Bioprospecting for Hydroxynitrile Lyases by Blue Native PAGE Coupled HCN Detection. *Curr. Biotechnol.* **4**, 111–117 (2015).

E. Lanfranchi, T. Pavkov-Keller, E. M. Koehler, et al., Enzyme discovery beyond homology: A unique hydroxynitrile lyase in the Bet v1 superfamily. *Sci. Rep.* **7**, 46738 (2017).

Asymmetric Synthesis of Optically Pure α-Substituted Carboxylic Acids

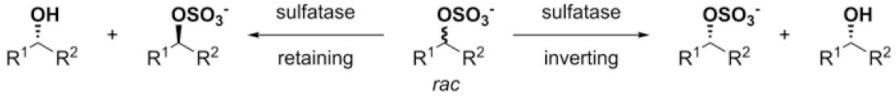


AMDase = arylmalonate decarboxylase

S. K. Gaßmeyer, J. Wetzig, C. Mügge, et al., Arylmalonate Decarboxylase-Catalyzed Asymmetric Synthesis of Both Enantiomers of Optically Pure Flurbiprofen. *ChemCatChem*. **8**, 916–921 (2016).

K. Miyamoto, R. Kourist, Arylmalonate decarboxylase—a highly selective bacterial biocatalyst with unknown function. *Appl. Microbiol. Biotechnol.* **100(20)**, 8621–8631 (2016).

Bio-Mitsunobu-Inversion



S. R. Wallner, M. Bauer, C. Würdemann, et al., Highly enantioselective sec-alkyl sulfatase activity of the marine planctomycete *Rhodopirellula baltica* shows retention of configuration. *Angew. Chem. Int. Ed.* **44(39)**, 6381–6384 (2005).

M. Schober, M. Toesch, T. Knaus, et al., One-pot deracemization of sec-alcohols: Enantioconvergent enzymatic hydrolysis of alkyl sulfates using stereocomplementary sulfatases. *Angew. Chem. Int. Ed.* **52(11)**, 3277–3279 (2013).

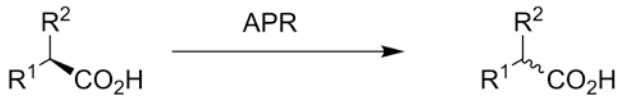
M. Fuchs, M. Toesch, M. Schober, et al., Chemoenzymatic asymmetric total synthesis of (R)-lasiodiplodin methyl ether through a sulfatase-based deracemization process. *European J. Org. Chem.* **2013**, 356–361 (2013).

M. Schober, K. Faber, Inverting hydrolases and their use in enantioconvergent biotransformations. *Trends Biotechnol.* **31**, 468–478 (2013).

M. Toesch, M. Schober, K. Faber, Microbial alkyl- and aryl-sulfatases: Mechanism, occurrence, screening and stereoselectivities. *Appl. Microbiol. Biotechnol.* **98(4)**, 1485–1496 (2014).

Enzymatic Isomerisation

Racemization of Arylpropionates



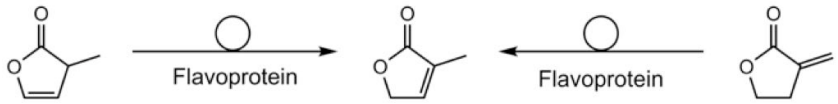
R^1 = aryl, vinyl
R^2 = Et, Me, OH, NH_2, F

APR = arylpropionate racemase

S. K. Gaßmeyer, H. Yoshikawa, J. Enoki, et al., STD-NMR-Based Protein Engineering of the Unique Arylpropionate-Racemase AMDase G74C. *ChemBioChem.* **16(13)**, 1943–1949 (2015).

F. Busch, J. Enoki, N. Hülsemann, et al., Semiempirical QM/MM calculations reveal a step-wise proton transfer and an unusual thiolate pocket in the mechanism of the unique arylpropionate racemase AMDase G74C. *Catal. Sci. Technol.* **6**, 4937–4944 (2016).

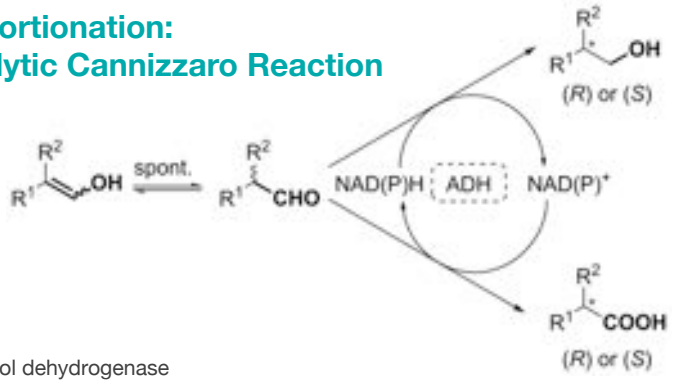
Isomerisation of C=C-Bond



K. Durchschein, S. Wallner, P. Macheroux, et al., Unusual C=C Bond Isomerization of an α,β-Unsaturated γ-Butyrolactone Catalysed by Flavoproteins from the Old Yellow Enzyme Family. *ChemBioChem.* **13(16),** 2346–2351 (2012).

N. G. Turrini, E. Eger, T. C. Reiter, et al., Sequential Enzymatic Conversion of α-Angelica Lactone to γ-Valerolactone through Hydride-Independent C=C Bond Isomerization. *ChemSusChem.* **9(24)**, 3393–3396 (2016).

Disproportionation: Biocatalytic Cannizzaro Reaction



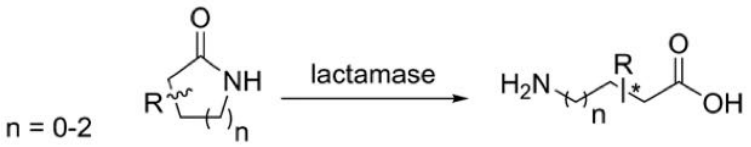
ADH = alcohol dehydrogenase

C. Wuensch, H. Lechner, S. M. Glueck, et al., Asymmetric biocatalytic cannizzaro-type reaction. *ChemCatChem.* **5(7)**, 1744–1748 (2013).

E. Tassano, K. Faber, M. Hall, Biocatalytic Parallel Interconnected Dynamic Asymmetric Disproportionation of α-Substituted Aldehydes: Atom-Efficient Access to Enantiopure (S)-Profens and Profenols. *Adv. Synth. Catal.* **360**, 2742–2751 (2018).

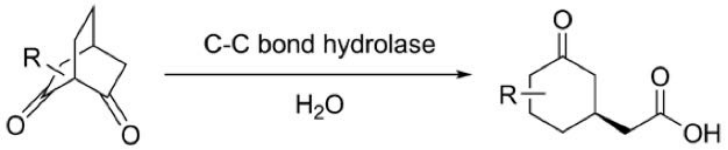
Hydrolysis / Esterification

Hydrolysis of Lactams



Z. Assaf, E. Eger, Z. Vitnik, et al., Identification and application of enantiocomplementary lactamases for Vince lactam derivatives. *ChemCatChem.* **6**, 2517–2521 (2014).

C-C Bond Hydrolysis

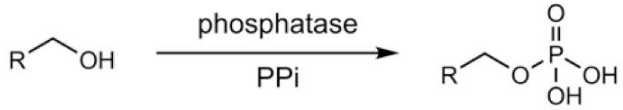


E. Siirola, A. Frank, G. Grogan, et al., C-C hydrolases for biocatalysis. *Adv. Synth. Catal.* **355**, 1677–1691 (2013).

E. Siirola, F. G. Mutti, B. Grischek, et al., Asymmetric synthesis of 3-substituted cyclohexylamine derivatives from prochiral diketones via three biocatalytic steps. *Adv. Synth. Catal.* **355**, 1703–1708 (2013).

A. Frank, E. Siirola, W. Kroutil, et al., Mutational analysis of the C-C bond cleaving enzyme phloretin hydrolase from Eubacterium ramulus. *Top. Catal.* **57**, 376–384 (2014).

Enzymatic Phosphorylation

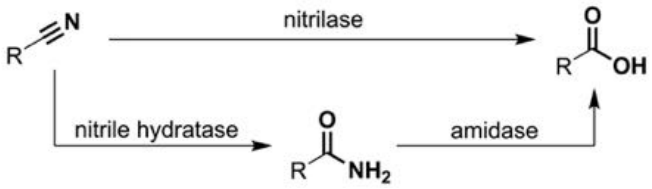


PPi = Pyrophosphate

G. Tasnádi, M. Lukesch, M. Zechner, et al., Exploiting Acid Phosphatases in the Synthesis of Phosphorylated Monoalcohols and Diols. *European J. Org. Chem.* **2016**, 45–50 (2016).

G. Tasnádi, W. Jud, M. Hall, et al., Evaluation of Natural and Synthetic Phosphate Donors for the Improved Enzymatic Synthesis of Phosphate Monoesters. *Adv. Synth. Catal.* **360**, 2394–2401 (2018).

Nitrile Hydrolysis



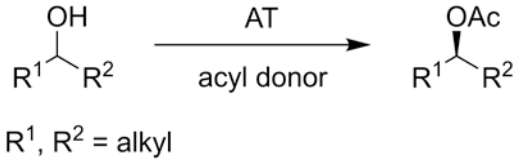
M. Winkler, L. Martínková, A. C. Knall, et al., Synthesis and microbial transformation of β-amino nitriles. *Tetrahedron.* **61**, 4249–4260 (2005).

U. Schreiner, B. Hecher, S. Obrowsky, et al., Directed evolution of *Alcaligenes faecalis nitrilase. Enzyme Microb. Technol.* **47**, 140–146 (2010).

U. Schreiner, G. Steinkellner, J. D. Rozzell, et al., Improved fitness of *Arabidopsis thaliana nitrilase* 2. ChemCatChem. **2**, 263–267 (2010).

B. Wilding, A. B. Veselá, J. J. B. Perry, et al., An investigation of nitrile transforming enzymes in the chemo-enzymatic synthesis of the taxol sidechain. *Org. Biomol. Chem.* **13**, 7803–7812 (2015).

Enantioselective Ester Formation in Aqueous Systems

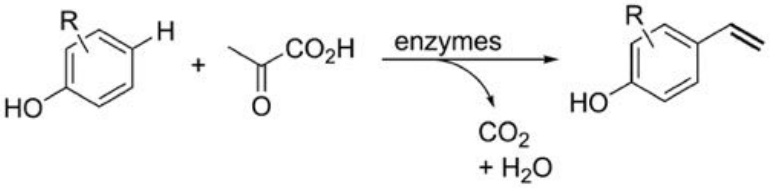


AT= acyl transferase

M. Kazemi, X. Sheng, W. Kroutil, et al., Computational Study of *Mycobacterium smegmatis* Acyl Transferase Reaction Mechanism and Specificity. *ACS Catal.* **8**, 10698–10706 (2018).

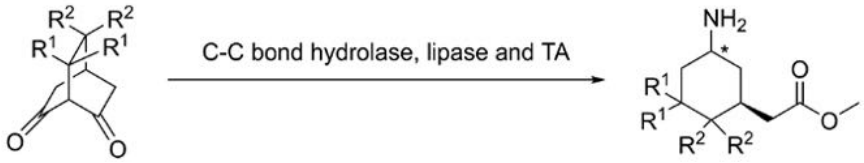
Enzymatic Cascades

Vinylation of Phenols



E. Busto, R. C. Simon, W. Kroutil, Vinylation of Unprotected Phenols Using a Biocatalytic System. *Angew. Chem. Int. Ed.* **54**, 10899–10902 (2015).

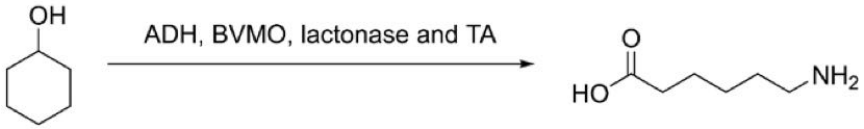
Cyclohexylamines from Diketones



TA = ω-transaminase

E. Siirola, F. G. Mutti, B. Grischek, et al., Asymmetric synthesis of 3-substituted cyclohexylamine derivatives from prochiral diketones via three biocatalytic steps. *Adv. Synth. Catal.* **355**, 1703–1708 (2013).

6-Aminohexanoic Acid from Cyclohexanol



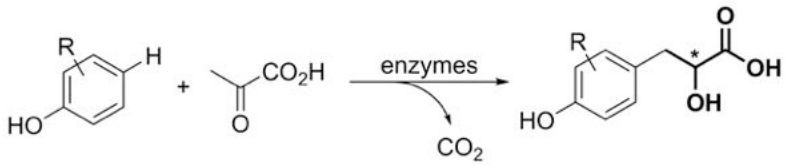
ADH = alcohol dehydrogenase; BVMO = Baeyer-Villiger monooxygenase; TA = ω-transaminase

R. C. Simon, N. Richter, E. Busto, et al., Recent developments of cascade reactions involving ω-transaminases. *ACS Catal.* **4**, 129–143 (2014).

J. H. Sattler, M. Fuchs, F. G. Mutti, et al., Introducing an *in situ* capping strategy in systems biocatalysis to access 6-aminohexanoic acid. *Angew. Chem. Int. Ed.* **53**, 14153–14157 (2014).

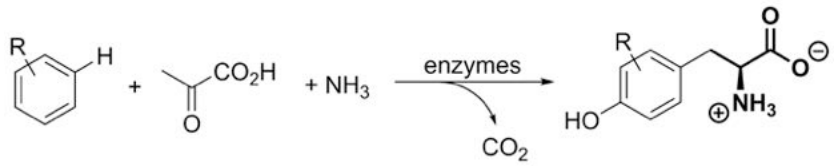
Enzymatic Cascades

Enantiopure Lactic Acids from Phenols



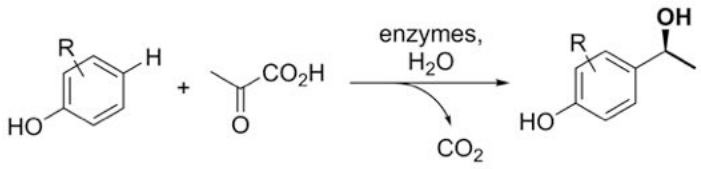
E. Busto, R. C. Simon, N. Richter, et al., One-Pot, Two-Module Three-Step Cascade to Transform Phenol Derivatives to Enantiomerically Pure (R)- or (S)-p-Hydroxyphenyl Lactic Acids. *ACS Catal.* **6(4)**, 2393–2397 (2016).

L-Tyrosine Derivatives from Benzenes



A. Dennig, E. Busto, W. Kroutil, et al., Biocatalytic One-Pot Synthesis of l-Tyrosine Derivatives from Monosubstituted Benzenes, Pyruvate, and Ammonia. *ACS Catal.* **5(12),** 7503–7506 (2015).

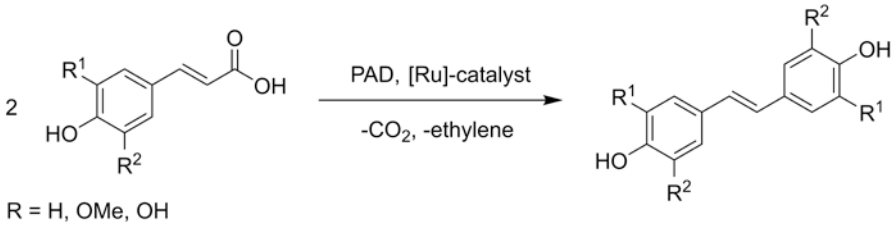
Hydroxyethylation of Phenols



S. E. Payer, H. Pollak, B. Schmidbauer, et al., Multienzyme One-Pot Cascade for the Stereoselective Hydroxyethyl Functionalization of Substituted Phenols. *Org. Lett.* **20(17)**, 5139–5143 (2018).

Enzymatic Cascades

Chemoenzymatic Preparation of Bio-Based Anti-Oxidants

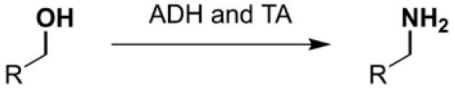


R = H, OMe, OH

PAD = phenolic acid decarboxylase

Á. Gómez Baraibar, D. Reichert, C. Mügge, et al., A One-Pot Cascade Reaction Combining an Encapsulated Decarboxylase with a Metathesis Catalyst for the Synthesis of Bio-Based Antioxidants. *Angew. Chem. Int. Ed.* **55**, 14823–14827 (2016).

Direct Amination of Alcohols

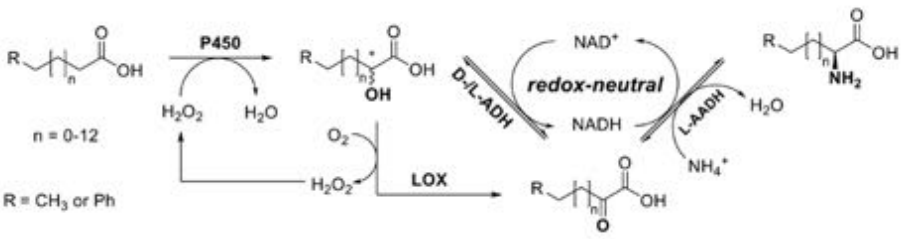


ADH = alcohol dehydrogenase; TA = ω-transaminase

R. C. Simon, N. Richter, E. Busto, et al., Recent developments of cascade reactions involving ω-transaminases. *ACS Catal.* **4**, 129–143 (2014).

J. H. Sattler, M. Fuchs, K. Tauber, et al., Redox self-sufficient biocatalyst network for the amination of primary alcohols. *Angew. Chem. Int. Ed.* **51**, 9156–9159 (2012).

Enantioselective α-Oxidation and Amination of Carboxylic Acids



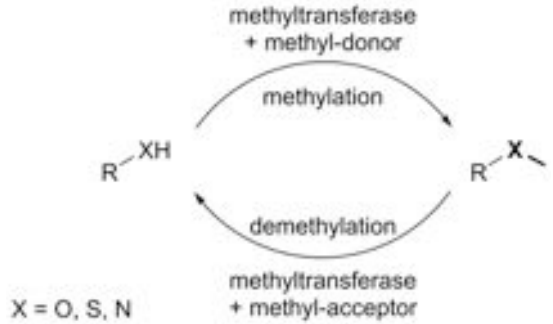
LOX = lactase oxidase; ADH = alcohol dehydrogenase; AADH = amino acid dehydrogenase

S. Gandomkar, A. Dennig, A. Dordic, et al., Biocatalytic Oxidative Cascade for the Conversion of Fatty Acids into α-Ketoacids via Internal H_2O_2 Recycling. *Angew. Chem. Int. Ed.* **52**, 427–430 (2018).

A. Dennig, S. Gandomkar, E. Cigan, et al., Enantioselective biocatalytic formal α-amination of hexanoic acid to l-norleucine. *Org. Biomol. Chem.* **16**, 8030–8033 (2018).

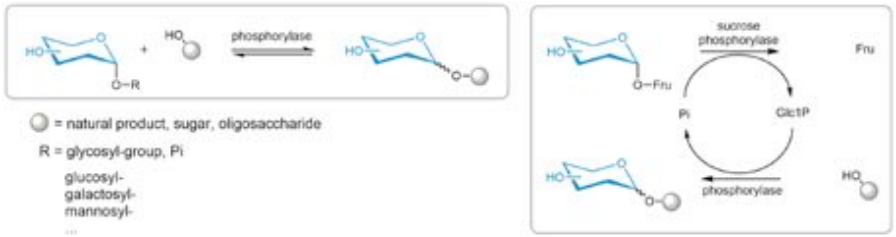
A. Dennig, F. Blaschke, S. Gandomkar, et al., Preparative Asymmetric Synthesis of Canonical and Non-canonical α-amino Acids Through Formal Enantioselective Biocatalytic Amination of Carboxylic Acids. *Adv. Synth. Catal.* (2019), doi:10.1002/adsc.201801377.

Methylation and De-Methylation



F. Zepeck, J. E. Farnberger, W. Skibar, et al., Biocatalytic methylation and demethylation via a shuttle catalysis concept involving corrinoid proteins. *Commun. Chem.* **1**, 82 (2018).

Phosphorylase Technology: Direct & Indirect Glucosylation

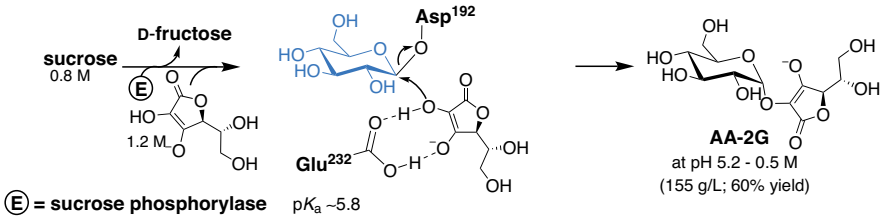


C. Goedl, T. Sawangwan, P. Wildberger, et al., Sucrose phosphorylase: A powerful transglucosylation catalyst for synthesis of α-D-glucosides as industrial fine chemicals. *Biocatal. Biotransformation.* **28:1**, 10–21 (2010).

C. Luley-Goedl, B. Nidetzky, Carbohydrate synthesis by disaccharide phosphorylases: Reactions, catalytic mechanisms and application in the glycosciences. *Biotechnol. J.* **5**, 1324–1338 (2010).

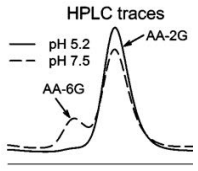
T. Desmet, W. Soetaert, P. Bojarová, et al., Enzymatic glycosylation of small molecules: Challenging substrates require tailored catalysts. *Chem. - A Eur. J.* **18**, 10786–10801 (2012).

ASCORBIC ACID 2-GLUCOSIDE

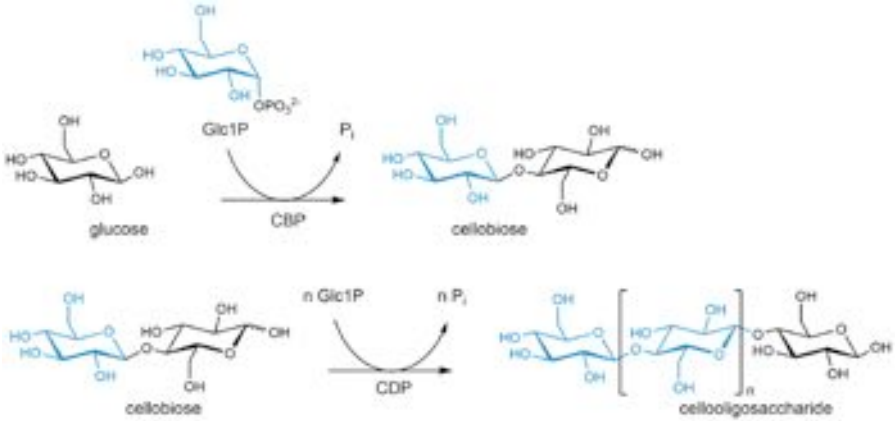


W02017050920 (2017)

R. K. Gudiminchi, B. Nidetzky, Walking a Fine Line with Sucrose Phosphorylase: Efficient Single-Step Biocatalytic Production of L-Ascorbic Acid 2-Glucoside from Sucrose. *ChemBioChem.* **18**, 1387–1390 (2017).



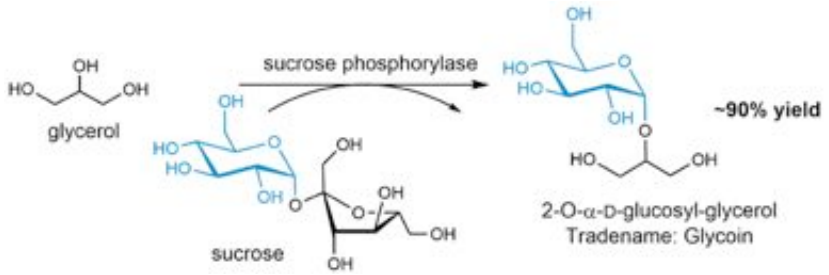
CELLO-OLIGOSACCHARIDES



CBP = cellobiose phosphorylase; CDP = cellodextrin phosphorylase

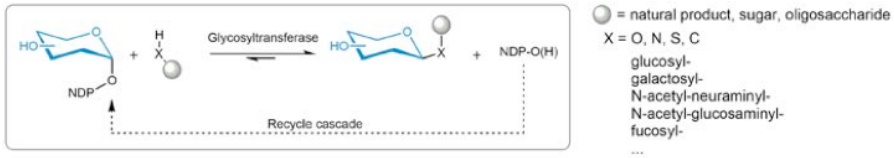
C. Zhong, C. Luley-Goedl, B. Nidetzky, *Biotechnol. Bioeng.*, in press.

GLUCOSYLGLYCEROL



C. Goedl, T. Sawangwan, M. Mueller, et al., A high-yielding biocatalytic process for the production of 2-O-(α-D-glucopyranosyl)-sn-glycerol, a natural osmolyte and useful moisturizing ingredient. *Angew. Chem. Int. Ed.* **47**, 10086–10089 (2008).

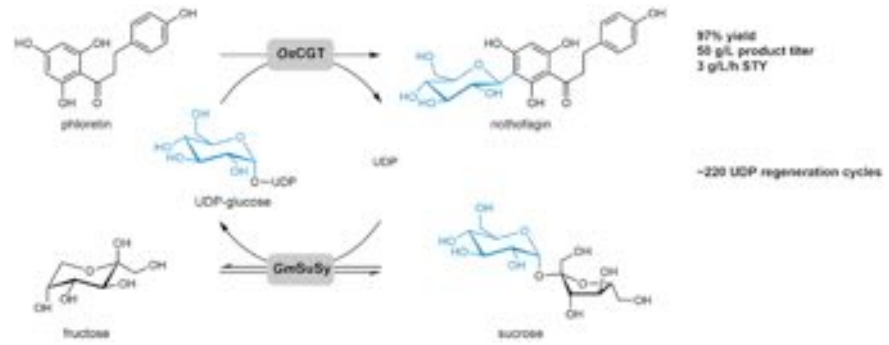
Glycosyltransferase Technology



A. Gutmann, B. Nidetzky, Enzymatic C-glycosylation: Insights from the study of a complementary pair of plant O- and C-glucosyltransferases. *Pure Appl. Chem.* **85**, 1865–1877 (2013).

B. Nidetzky, A. Gutmann, C. Zhong, Leloir Glycosyltransferases as Biocatalysts for Chemical Production. *ACS Catal.* **8**, 6286–6300 (2018).

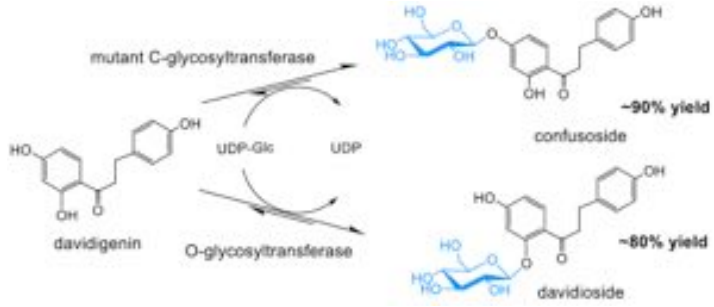
NOTHOFAGIN



SuSy = sucrose synthase; CGT = C-glucosyltransferase

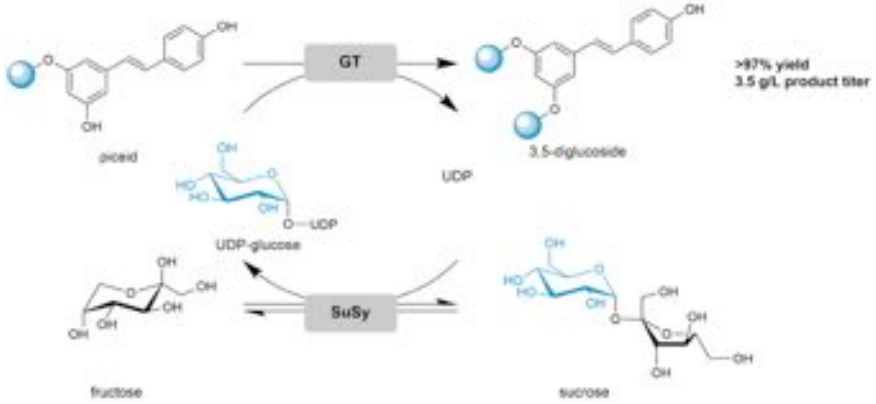
K. Schmölzer, M. Lemmerer, B. Nidetzky, Glycosyltransferase cascades made fit for chemical production: Integrated biocatalytic process for the natural polyphenol C-glucoside nothofagin. *Biotechnol. Bioeng.* **115**, 545–556 (2018).

DIHYDROCHALCONE GLUCOSIDES



A. Gutmann, L. Bungaruang, H. Weber, et al., Towards the synthesis of glycosylated dihydrochalcone natural products using glycosyltransferase-catalysed cascade reactions. *Green Chem.* **16**, 4417–4425 (2014).

RESVERATROL 3,5-β-D-GLUCOSIDE

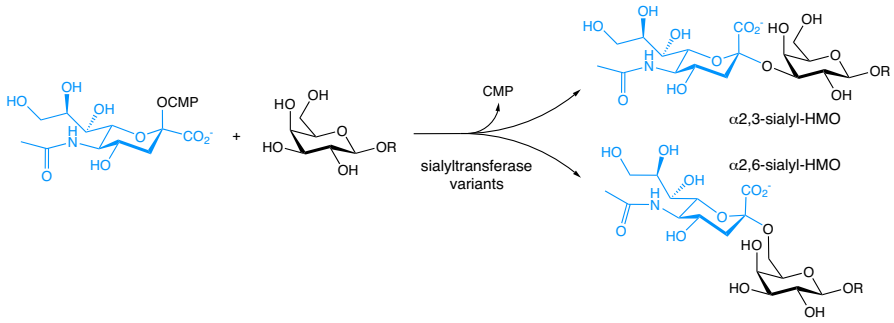


SuSy = sucrose synthase; GT = glycosyltransferase

A. Lepak, A. Gutmann, S. T. Kulmer, et al., Creating a Water-Soluble Resveratrol-Based Antioxidant by Site-Selective Enzymatic Glucosylation. *ChemBioChem.* **16**, 1870–1874 (2015).

Regioselective Glycosyl Transfer

3’- and 6’-sialyl-HMOs

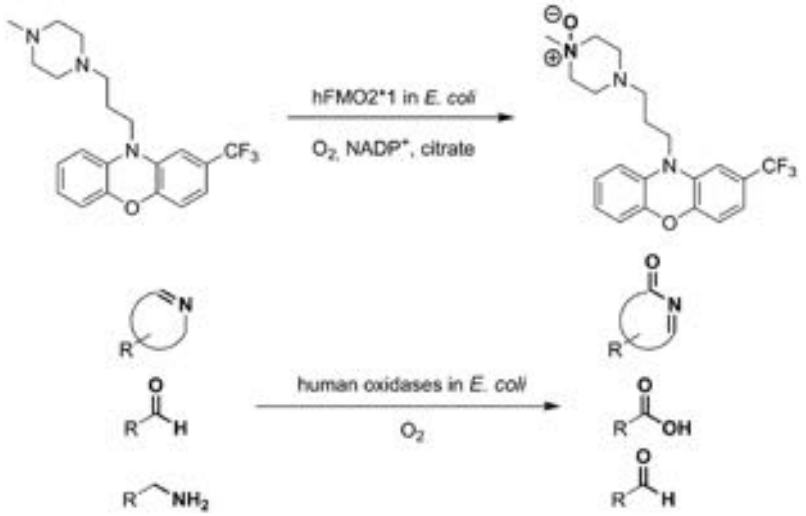


R = rest of acceptor oligosaccaride (e.g. glucose Glc, *N*-acetylglucosamine GlcNAc)
Lactose (R = Glc), *N*-acetyllactosamine (R = GlcNAc)

K. Schmölzer, T. Czabany, C. Luley-Goedl, et al., Complete switch from α-2,3- to α-2,6-regioselectivity in *Pasteurella dagmatis* β-d-galactoside sialyltransferase by active-site redesign. Chem. Commun. **51**, 3083–3086 (2015).

API-Modification with Human Enzymes

Chemo- & Regioselective Oxidation of Soft Nucleophiles



hFMO2 = human flavin-containing monooxygenase 2

S. P. Hanlon, A. Camattari, S. Abad, et al., Expression of recombinant human flavin monooxygenase and moclobemide-N-oxide synthesis on multi-mg scale. *Chem. Commun.* **48**, 6001–6003 (2012).

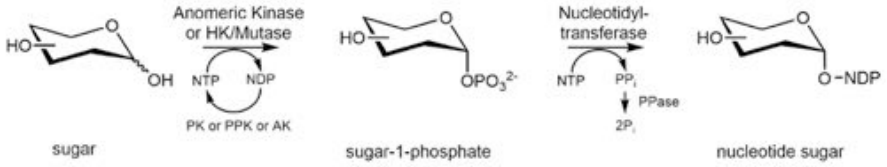
D. Rodrigues, M. Kittelmann, F. Eggimann, et al., Production of Recombinant Human Aldehyde Oxidase in *Escherichia coli* and Optimization of Its Application for the Preparative Synthesis of Oxidized Drug Metabolites. *ChemCatChem.* **6**, 1028–1042 (2014).

M. Geier, T. Bachler, S. P. Hanlon, et al., Human FMO2-based microbial whole-cell catalysts for drug metabolite synthesis. *Microb. Cell Fact.* **14**, 82 (2015).

M. Ferreira Antunes, F. K. Eggimann, M. Kittelmann, et al., Human xanthine oxidase recombinant in *E. coli*: A whole cell catalyst for preparative drug metabolite synthesis. *J. Biotechnol.* **235**, 3–10 (2016).

M. Winkler, M. Geier, S. P. Hanlon, et al., Human Enzymes for Organic Synthesis. *Angew. Chemie Int. Ed.* **57**, 13406–13423 (2018).

Kinase & Transferase

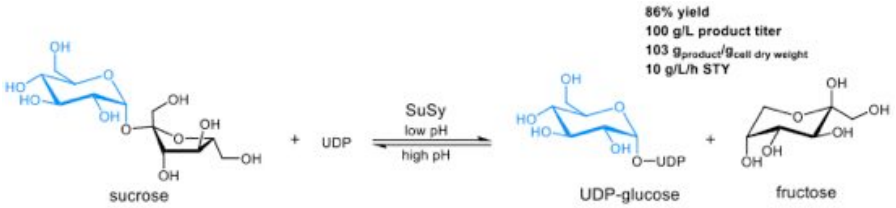


HK = hexokinase, PK = pyruvate kinase; PPK = polyphosphate kinase; AK = acetate kinase; ManC = mannose-1-phosphate guanylyltransferase; PPase = pyrophosphatase

Examples synthesized: GDP-L-fucose, GDP-mannose, UDP-glucose, UDP-galactose, UD-P-glucuronic acid, various rare sugar nucleotides

Synthase

UDP-GLUCOSE

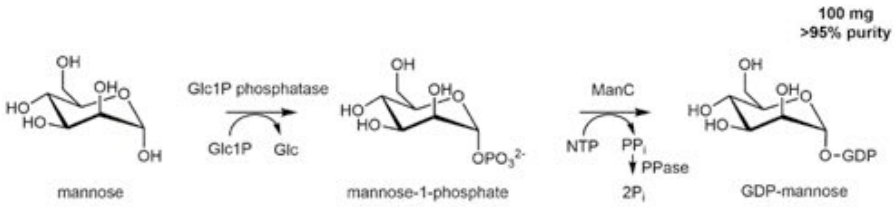


SuSy = sucrose synthase

K. Schmölzer, M. Lemmerer, A. Gutmann, et al., Integrated process design for biocatalytic synthesis by a Leloir Glycosyltransferase: UDP-glucose production with sucrose synthase. *Biotechnol. Bioeng.* (2017), doi:10.1002/bit.26204.

Phosphatase & Transferase

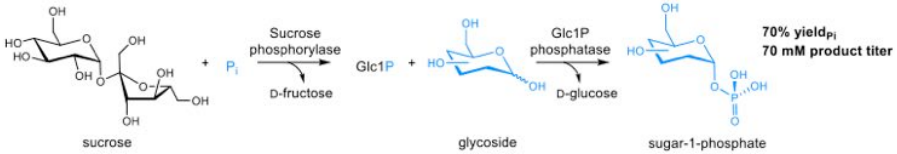
GDP-MANNOSE



M. Pfeiffer, D. Bulfon, H. Weber, et al., A Kinase-Independent One-Pot Multienzyme Cascade for an Expedient Synthesis of Guanosine 5′-Diphospho-d-mannose. *Adv. Synth. Catal.* **358**, 3809–3816 (2016).

Phosphorylase & Phosphatase

SUGAR-1-PHOSPHATES



P. Wildberger, M. Pfeiffer, L. Brecker, et al., Diastereoselective Synthesis of Glycosyl Phosphates by Using a Phosphorylase-Phosphatase Combination Catalyst. *Angew. Chem. Int. Ed.* **54**, 15867–15871 (2015).

In-silico Search for Novel Biocatalysts

Traditional screening for novel enzymes requires time-consuming experiments and expensive activity assays in the wet-lab. To reduce costs, the prediction and identification of enzyme functionalities is a major challenge of modern bioinformatics. However, the computational annotation of proteins proves to be difficult erroneous and lacks the possibility to identify completely independent novel biocatalysts because they rely on the correlation of (sequence) similarities with the known functions of the template and are bound to find „more of the same".

CATALOPHORE SEARCH FOR NOVEL ENZYMES

acib-researchers developed a patented bioinformatics method to mine structural databases using three dimensional search templates which cover the arrangement of chemical functional groups or pre-calculated point-clouds representing the „empty space" of active sites. These search templates are termed „catalophores" (i.e. carrier of the catalytic function). The searches are independent of structural or sequence similarities to currently employed enzymes. Therefore, these identified enzymes may feature different physico-chemical properties such as stability selectivity or substrate tolerance.
A successful test-case led to the identification of two „novel" ene-reductases, by searching with patterns obtained from classical old yellow enzymes. The identified enzymes showed significant conversions on typical old yellow enzyme substrates and even allowed access to enantiomers that could not be obtained using current enzyme portfolio although the overall sequence and structural similarity are below 10 %.

G. Steinkellner, C. C. Gruber, T. Pavkov-Keller, et al., Identification of promiscuous ene-reductase activity by mining structural databases using active site constellations. *Nat. Commun.* **5**, 4150 (2014).

The Austrian Centre of Industrial Biotechnology

ABOUT ACIB

The Austrian Centre of Industrial Biotechnology (acib) is a top-class international research institution in the field of industrial biotechnology. Since its foundation in 2010 the centre of has specialized on the development of innovative, eco-friendly and economical processes for the biotech-, chemical- and pharmaceutical industries using the methods and tools of nature. Presently 200+ researchers are carrying out research in more than 125 research projects.

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