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Reviews

Concepts of Nature in Organic Synthesis: Cascade Catalysis and Multistep Conversions in Concert

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Abstract:

The development of cascade conversions (i.e. combined (catalytic) reactions without intermediate recovery steps as taking place in living cells) is considered as one of the important future directions for carrying out sustainable organic syntheses with inherently safer designs. It will drastically reduce operating time and costs as well as consumption of auxiliary chemicals and use of energy. Production of waste for multistep syntheses might ultimately reach the level of well below 1 kg per kg of product. The concept of cascade conversions is demonstrated by a representative selection of illustrative, mostly catalytic, examples on laboratory as well as on pilot or industrial scale and discussed within the strategic context of new synthesis methodology as required by present developments in life sciences industries. Full exploitation of such multistep syntheses and catalysis will require the development of novel, mutually compatible, organic and biosynthetic methods and procedures. Eventually a full integration of organic synthesis and biosynthesis can be envisaged. Syntheses requiring a smaller range of reaction parameters and reagents but a more intricate array of efficient catalysts might be expected. Integration with in situ product separation techniques will become mandatory, preferably by continuous processing.

Introduction

Mankind's Synthesis Efforts. Quite different from the petrochemical industry, stoichiometric synthesis has for many years been a satisfactory tool for the preparation of molecules for application in life science industries. Timely introduction of biocatalysis has allowed organic synthesis in the fine chemical industry to meet both higher demands in molecular complexity of its products and a better eco-efficiency in its processes.

However, organic synthesis, i.e., chemistry by mankind, still uses often a simple step-by-step approach to convert a starting material **A** into a final product **D**, in which intermediate products **B** and **C** are isolated and purified for each next conversion step (Figure 1). Catalytic steps are mostly combined with stoichiometric steps in the preparation of precursors or in the further downstream processing.

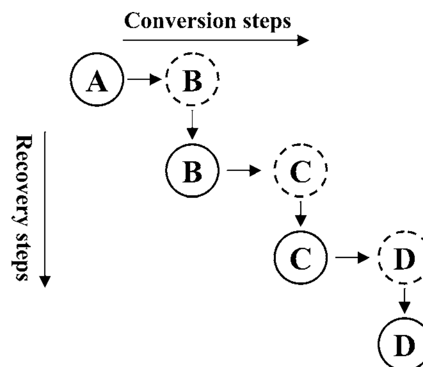

Figure 1. **Chemistry as it is done in the laboratory or manufacturing plant traditionally involves a recovery step after each conversion step.**

Obvious disadvantages are low space−time yields (kg/L/h), laborious recycle loops and large amounts of waste.[1] Moreover, time-consuming route development and scale-up are increasingly hampering a much desired shortening of time to market of the end products.

High-throughput screenings and combinatorial chemistry have greatly expanded the generation of lead compounds in life science industries, stimulated also by the impact of modern molecular biology and the fast developments in genomics. Most of these lead compounds, however, are prepared using stoichiometric chemistry. Many small- and medium-sized companies, active in producing larger amounts (from gram- to kilogram-scale), also resort to the great diversity of organic syntheses in constructing their molecules. Great advances are made in both biocatalysis and chemocatalysis, in particular with respect to asymmetric synthesis, but processes are still mainly step-by-step and rather arbitrary combinations of catalytic and stoichiometric conversions.[2] The latter are posing increasing problems for an ecoefficient and economic manufacturing at plant scale.

Nature's Synthesis Efforts. Biosynthesis, i.e. chemistry by Nature, in the cells of living organisms, goes through a multistep-cascade approach to convert a starting material **A** into the final product **D** without separation of intermediates **B** and **C** (Figure 2). In these reaction sequences, concentrations of starting materials, intermediates, and end products

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(1) Bruggink, A. *Chim. Oggi* **1998**, *September*, 44.
(2) (a) Schoevaart, R.; Kieboom, T. *Chem. Innovation* **2001**, *12*, 33. (b) Schoevaart, R.; Kieboom, T. Trends in Drug Research III. In *Pharmacochem. Libr.* **2002**, *32*, 39.

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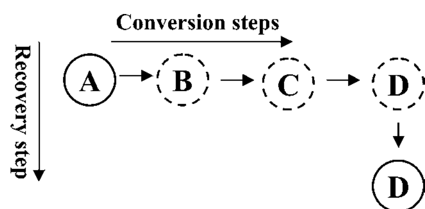

Figure 2. **Chemistry as it is done in the cells of organisms involves coupled conversions without intermediate recovery steps.**

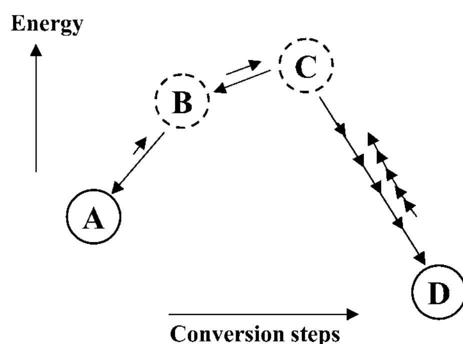

Figure 3. **Potential power of cascade conversions to overcome thermodynamic hurdles in multistep syntheses.**

are kept very low, allowing maximum selectivity, no side reactions, and no formation of byproducts. Starting materials are coming in according to fed-batch principles using controlled membrane transport proteins. End products are removed using the in situ product removal (ISPR) principle, again employing controlled transport systems. This allows, in principle, high throughputs and efficiencies. Required activating and protecting groups are kept in situ through recyclable energy and redox carriers (ATP, NADP, etc.).

Such multistep concerted syntheses are quite common in everyday life, for example in saving energy to overcome eventual thermodynamic hurdles to reach the final product **D** in case of higher-energetic intermediates **B** and/or **C**, as demonstrated here for the well-known glycolysis pathway (Figure 3). The equilibria between fructose 1,6-diphosphate (**A**), dihydroxyacetone phosphate (**B**), and glyceraldehyde 3-phosphate (**C**) are quite unfavourable: [**A**]:[**B**]:[**C**] > 1000: 20:1. Nevertheless, complete transformation of **A** to lactic acid (**D**) occurs through a coupled multienzymatic conversion of the more highly energetic **C** intermediate. In addition, the success of this overall cascade conversion is brought about by the very fast aldolase- and isomerase-catalysed equilibria, **A** $\leftrightarrows$ **B** and **B** $\leftrightarrows$ **C**, respectively.[3]

Integrated Synthesis Efforts. Although beyond the scope of the present report, metabolic engineering of microorganisms promises powerful means to convert part of the traditional multistep organic syntheses into one-step fermentation processes. For example, a substantial waste and energy reduction has been achieved very recently by DSM anti-infectives on an industrial scale through the integration of part of the former chemical steps towards 7-ADCA, the key intermediate for a number of cephalosporins such as the antibiotic cephalexin, in a single fermentation step.[4] An increasing range of fully fermentative processes for non-natural products might be expected.

As biotechnology, however, cannot replace all multistep chemical process routes, nature-inspired organic chemical syntheses have to be developed, too. Therefore, for the next generation of organic synthesis, it is the challenge to:

(1) combine the power of the chemical, chemocatalytic, enzymatic, and microbial conversions;

(2) search for multistep conversions without recovery steps such as nature does, i.e. to go for simultaneous or consecutive (multicatalytic) clean procedures;

(3) fine-tune reaction conditions and catalytic systems to allow for the right concerted cooperation without any intermediate isolation or purification steps;

(4) develop alternative conversion procedures that are mutually compatible with respect to the required reaction conditions, i.e. applicable without special precautions in one-pot concerted or consecutive multistep syntheses.

As an illustrative academic example, the potential power of such a combined catalytic approach, leading to both a 50-fold waste decrease and a 5-fold reduction of both operating and space−time, has been recently proven by us (cf entry **47** of Table 1). We performed a three-step carbohydrate conversion using the consecutive action of an enzyme, a homogeneous and a heterogeneous catalyst in water. No protective groups were used, and intermediate recovery steps were not needed (Figure 4). Thus, cascade conversions can be seen as a potentially important tool for more sustainable organic syntheses in the practice of the fine chemical industry.

Review of Different Types of Cascade Conversions. Both literature and patents show an increasing number of cascade reactions. The vast majority deals with the concept of one-pot reactions. Both bio- and chemocatalysis as well as stoichiometric organic chemistry can work together. Compatibility and compartmentalization are key concepts in developing cascade syntheses. The living cell is furnishing an increasing insight for developing new concepts for cascade conversions. A first, tentative conclusion can be made immediately: development of one-pot cascades in homogeneous systems is searching for exceptions, whereas a clever design of compartmentalization might lead to new concepts.

A variety of promising cascade conversions have been described in the literature, involving different combinations of enzymes, homogeneous and heterogeneous catalysts, and of uncatalyzed organic chemical conversions. A representative selection of a number of illustrative, mostly catalytic, examples on laboratory as well as on pilot or industrial scale has been collected in Tables 1 and 2, respectively. The different approaches have been categorized in bio−bio (entries **1**−**21** and **63**−**64**), bio−chemo (entries **22**−**47** and **65**−**67**), and chemo−chemo (entries **48**−**62** and **68**−**73**) cascades that have been arranged in increasing order of number of transformations, thus giving the concept of cascade conversions a more defined form.

(3) Lehninger, A. L. *Principles of Biochemistry*; Worth Publishers: New York, 1984; p 397.

(4) DSM Anti-infectives. *DSM Magazine* **1998**, *147*, 18.

Table 1. **Cascade conversions from milligram to multigram laboratory scale**

conversions number of transformations [chemo + bio = total]	reaction type and conditions	catalysts and/or reagents	additional comments	refs
	Bio−Bio (Entries 1−21)			
acetoacetate ester + D-glucose → (*R*)-3-hydroxybutanoate ester + D-gluconate [0 + 2 = 2] **1**	reduction cofactor-regeneration [two-phase system ambient conditions]	glucose dehydrogenase aldehyde dehydrogenase	combined two-enzymatic action in one pot for enantioselective carbonyl reduction, using D-glucose as the reducing agent	5
α-keto acids + NH_3 + NADH → D- or L-amino acids + NAD coupled with: formate + NAD → NADH + CO_2 or: D-glucose + NAD → NADH + D-gluconic acid [0 + 2 = 2] **2**	reductive amination cofactor regeneration [water, ambient conditions]	amino acid dehydrogenases formate dehydrogenase or: glucose oxidases	combined two-enzymatic action in one pot with the use of either formate or D-glucose as reducing agent, giving (unnatural) amino acids in high yields and ee's	6 7 8
α-keto acids + small amino acid → D- or L-amino acids + small α-keto acid coupled with: reduction or decomposition of the small α-keto acid [0 + 2 = 2] **3**	transamination small α-keto acid conversion	amino acid aminotransferase formate dehydrogenase	one-pot, two-enzyme reductive enantioselective amination, using small amino acids as the amination agent	9
starch + H_2O → D-glucose ⇔ D-fructose [0 + 2 = 2] **4**	acetal hydrolysis aldo−keto isomerization [double-immobilized enzyme system water, pH = 6, *T* 70 °C]	glucoamylase glucose isomerase	the amylase enzyme was chemically coupled to polymer-immobilized isomerase enzyme, giving after 24 h twice the amount of fructose−glucose syrup as compared to a mixture of the free enzymes	10
sucrose + O_2 → D-fructo-oligosaccharides + D-gluconic acid [0 + 2 = 2] **5**	fructosyl transfer glucose oxidation [water, pH 6, *T* 50 °C]	fructosyl transferase glucose oxidase	combined two-enzymatic reaction in one pot; oxidation of D-glucose is the extra driving force for the oligomerization, raising the yield from 50 to 60% to as high as 90%	11
polyunsaturated triacylglycerols + H_2O → polyunsaturated fatty acids + glycerol + O_2 → hydroperoxypolyunsaturated fatty acids + glycerol [0 + 2 = 2] **6**	hydrolysis peroxidation [octane/water pH 9, *T* 26 °C]	lipase lipoxygenase	fat hydrolysis coupled with fatty acid oxidation in a two-phase, one-pot system, yielding up to 96% hydroperoxides after 5.5 h reaction time	12
PhCOCOOH + NADH → (*R*)-mandelic acid + NAD coupled with: formate + NAD → CO_2 + NADH [0 + 2 = 2] **7**	ketone reduction cofactor regeneration	mandelic acid dehydrogenase formate dehydrogenase	continuous conversion in an enzyme membrane reactor; high space−time yields of 30 $g \cdot L^{1} \cdot h^{-1}$ with low enzyme consumption	13 14 15
4-methylcylcohexanone + O_2 + NADH → (*S*)-4-methylcaprolacton + NAD + H_2O coupled with: formate + NADP → CO_2 + NADPH [0 + 2 = 2] **8**	oxidation cofactor regeneration [water, pH 8, 30 °C]	cyclohexanone monooxygenase formate dehydrogenase	combined enzymatic action for enantioselective ε-lactone (>99% ee) formation in one pot on mmole scale	16
aldehyde + HCN → (*S*)-hydroxynitrile + H_2O → (*S*)-α-hydroxy amide or: aldehyde + HCN → (*S*)-hydroxynitrile + 2 H_2O → (*S*)-α-hydroxy acid [0 + 2 = 2] **9**	cyanohydrin formation nitrile hydratation or: cyanohydrin formation nitrile hydrolysis [water, ambient conditions]	hydroxynitrile lyase nitrile hydratase or: hydroxynitrile lyase nitrilase	two-enzyme one-pot approach for conversion of in situ formed cyanohydrins into enantiopure amino acid amides or amino acids of high enantiomeric purity	17 18

Table 1. (Continued)

conversions number of transformations [chemo + bio = total]	reaction type and conditions	catalysts and/or reagents	additional comments	refs
		Bio−Bio (Entries 1−21) (Continued)		
D-phenylglycine nitrile + H_2O → D-phenylglycine amide + 7-ADCA → cephalexin + NH_3 [0 + 2 = 2] **10**	nitrile hydration transamidation [water, ambient conditions]	nitrile hydratase pen acylase	direct coupling of aminonitriles in β-lactam antibiotics synthesis	19
D-glucose + O_2 → D-gluconic acid + H_2O_2 coupled with: thioanisole + H_2O_2 → (*R*)-methylphenyl sulfoxide [0 + 2 = 2] **11**	glucose oxidation enantioselective sulfoxidation [water, ambient conditions]	glucose oxidase chloroperoxidase	enzymatic formation and in situ use of hydrogen peroxide for enantioselective thioether oxidation	20
(*R*)-α-amino acid ester ⇔ (*S*)-α-amino acid ester + AcOR → (*S*)-α-*N*-acylamino ester + ROH [0 + 2 = 2] **12**	racemization esterification [organic solvent]	racemase lipase	100% ee/100% yield concept by one-pot combined enzymatic racemization and esterification in organic solvents	21 22
^{13}C-formate + glycine → 3-^{13}C-L-serine coupled with: D-glucose 6-phosphate + O_2 → D-gluconic acid 6-phosphate [0 + 3 = 3] **13**	hydroxymethylation cofactor regeneration oxidation [water, pH 7, *T* 30 °C]	serinehydroxy- methyltransferase dihydrofolate reductase glucose 6-phosphate dehydrogenase [dihydrofolate]	combined triple enzymatic conversion on 0.1-g scale in one pot, in 72% yield; applied for selective isotope labeling, thus avoiding multistep organic synthesis	23
α-ketoacid + NH_3 →→→ D-amino acid coupled with: formate + NAD → CO_2 + NADH [0 + 4 = 4] **14**	racemization reductive amination cofactor regeneration	alanine racemase L-alanine dehydrogenase D-amino acid aminotransferase formate dehydrogenase [pyridoxal phosphate]	range of α-ketoacids were converted on 0.1 mmol scale in 8 h into the corresponding D-amino acids with 70−98% yields; in most cases ee's of 90−100% were obtained	24
glycerol + PP_i → glycerol phosphate + O_2 → dihydroxyacetone phosphate + butanal → 5-deoxy-5-ethyl-D-xylulose 1-phosphate + H_2O → 5-deoxy-5-ethyl-D-xylulose + P_i [0 + 4 = 4] **15**	phosphorylation oxidation aldol reaction dephosphorylation [glycerol/water, *T* 25°C pH 4 → 7.5 → 4]	phytase oxidase + catalase aldolase phytase	subsequent four-step enzymatic conversion in one pot by pH switching enzymes on and off; dual action of phytase, i.e. phosphorylation and hydrolysis at 95% and 55% glycerol, respectively; overall yield 57%; broadly applicable for ketose syntheses	25
sugars →→→→→ (branched) carbohydrates and glycoconjugates [0 + <8 = ≤ 8] **16**	glycosidic bond formation epimerisation phosphorylation glycosyl transfer etc. cofactor regeneration e.g. by phosphoenolpyruvate [water, ambient conditions]	transferases phosphorylases epimerases etc. [several cofactors]	various proofs of principle for multienzyme one-pot strategies (on gram scale); phosphoenolpyruvate and diphosphate are the driving forces for the overall conversion (energy sources)	26
D-glucose → → → → → → riboflavin coupled with: PEP + ADP → pyruvate + ATP and: 2-ketoglutarate + NADPH → glutamate + NADP [0 + 8 = 8] **17**	phosphorylation oxidation reductive decarboxylation deformylation cyclyocondensation cofactor regenerations [water, ambient conditions]	hexokinase dehydrogenases (2×) synthases (3×) pyruvate kinase glutamate dehydrogenase	one-pot eight-enzyme synthesis of riboflavin, consisting of six enzymatic synthesis steps and two cofactor regeneration steps, with overall yields of 35−50%; energy is delivered by both phosphoenolpyruvate and 2-ketoglutarate	27

Table 1. (Continued)

conversions number of transformations [chemo + bio = total]	reaction type and conditions	catalysts and/or reagents	additional comments	refs
		Bio−Bio (Entries 1−21) (Continued)		
D-glucose 1-phosphate + UTP → PP_i+ UDP-D-glucose → UDP-D-galactose + *N*-acetyl-D-glucosamine → *N*-acetyllactosamine + [in situ: sialic acid + CTP → CMP-sialic acid + PP_i] → sialyl *N*-acetyllactosamine coupled with: phosphoenolpyruvate + UDP → pyruvate + UTP and: phosphoenolpyruvate + ADP → pyruvate + ATP and: phosphoenolpyruvate + CMP → pyruvate + CTP and: PP_i + H_2O → 2 P_i [0 + 8 = 8] **18**	UDP-ylation alcohol epimerization glycosylation phosphorylation hydrolysis cofactor regenerations [water, pH 7, *T* 25 °C]	UDP-glucose pyrophosphorylase UDP-glucose epimerase galactosyl transferase sialyltransferase nucleoside monophasphate kinase CMP-sialic acid synthetase pyruvate kinase pyrophosphatase [UDP; CMP; ATP]	eight-enzyme one-pot reaction on 10 g scale; phospho-enol-pyruvate and diphosphate are the driving force for the overall reaction (energy sources); many other examples of multienzyme one-pot carbohydrate syntheses are described and have been reviewed	28 29 30
sucrose → D-fructose + D-glucose ⇔ D-fructose + ATP → D-fructose 6-phosphate + ATP → D-fructose 1,6-diphosphate → D-glyceraldehyde 3-phosphate + dihydroxyacetone phosphate ⇔ dihydroxyacetone phosphate + RCHO → R-CHOH-CHOH-CO-$CH_2OPO_3^{2-}$ coupled with: ADP + phosphoenol pyruvate → ATP + pyruvate [0 + 8 = 8] **19**	acetal hydrolysis aldo−keto isomerization phosphorylation phosphorylation retro-aldol reaction aldo−keto isomerization retro-aldol reaction aldol reaction cofactor regeneration [water, pH 7, *T* 25 °C 0.1 M sucrose]	invertase glucose isomerase hexokinase fructose 6-phosphate kinase triose phosphate isomerase fructose 1,6-diphosphate aldolase pyruvate kinase [ATP; phosphoenol pyruvate]	multienzymatic one-pot syntheses of enantiopure unnatural ketose 1-phosphates, on gram scale, from 0.1 M sucrose solution; the phosphoenolpyruvate is the energy source for the overall conversion; reaction times up to 3 days, yields vary from 50 to 100%; a number of related examples have been described and reviewed	26 31
pol− + mono/disaccharides→ pol−mono/disaccharides → → → → pol−oligosaccharides + H_2O → pol− + oligosaccharides [0 + <10 = <10] **20**	glycosylation glycosyl transfer hydrolysis [water, ambient conditions]	galactosyl transferase sialyl transferase fucosyl transferase chymotrypsin phosphodiesterase [reagents for polymer attachment]	polymer-supported chemo-enzymatic synthesis, also applied to branched oligosaccharides, glycoconjugates, peptides, and oligonucleotides	32
δ-aminolevulinic acid + *S*-adenosyl methionine + O_2 + NADPH → → → → → → → → → → hydrogenobyrinic acid (HBA, the corrin moiety of vitamin B_{12}) [0 + 12 = 12] **21**	condensation dehydration deamination ring closure methylation oxidation ring contraction methylation elimination + methylation reduction decarboxylation methyl transfer [water, ambient conditions]	dehydratase deaminase porphyring synthase methyltransferases oxidase decarboxylase methylmutase [NADPH]	presenting the first 12 steps in one pot of the microscale 17-steps synthesis of vitamin B_{12} with over 90% yield per step (overall yield 20%); cascade record holder in vitro	33
		Bio−Chemo (Entries 22−47)		
D-glucose ⇔ D-fructose + H_2 → D-mannitol [1 + 1 = 2] **22**	aldo−keto isomerization [mutarotation] hydrogenation [water, pH 7.5, *T* 70 °C 50 atm H_2; traces of EDTA present to protect the enzyme against Cu^{2+}]	glucose isomerase [base] copper [borate]	100% de/100% yield concept by combined bio−chemo catalytic action in autoclave; traces of borate enhance hydrogenation selectivity to D-mannitol; D-mannitol yield of 65%, twice as high as by traditional process	34

Table 1. (Continued)

conversions number of transformations [chemo + bio = total]	reaction type and conditions	catalysts and/or reagents	additional comments	refs
	Bio−Chemo (Entries 22−47) (Continued)			
4-phenyl-2-butanone + $NADH^{mimic}$ → (*S*)-4-phenyl-2-butanol + NAD^{mimic} coupled with: formate + $NADH^{mimic}$ → CO_2 + $NADH^{mimic}$ [1 + 1 = 2] **23**	ketone reduction cofactor regeneration [pH 7.0, 30 °C]	organorhodium hydride horse liver alcohol dehydrogenase	combined bio−chemo catalytic action in one pot; Rh-hydride catalyst regenerates the cofactor mimic, the enzyme reduces the ketone	35
(*R*/*S*)-epoxide + H_2O → (*S*)-epoxide + (*S*)-glycol + H_2O → (*S*)-glycol [1 + 1 = 2] **24**	enantioselective epoxide hydrolysis stereoselective epoxide hydrolysis [water/dioxane or DMF *T* 30 °C → 10 °C, pH 7.5 → 1]	epoxide hydrolase acid	subsequent enzymatic and mineral acid catalyzed hydrolysis that occurs with retention and inversion of configuration, respectively, gives glycols in yields up to 98% with ee's up to 99%.	36 37 38
(*S*)-1,2-epoxy-3-bromopropane ⇔ (*R*)-1,2-epoxy-3-bromopropane + N_3^- → (*R*)-3-azido-3-bromo-2-propanol [1 + 1 = 2] **25**	racemization nucleophilic N_3^- addition [water, pH 6.5, *T* 30 °C]	Br^- alcohol dehalogenase	100% ee/100% yield concept by Br^- mediated epoxide racemization coupled with dynamic kinetic resolution of enzymatic azidolysis of the epoxide; on 0.5 g scale, yields up to 80%, ee's up to 99%	39
(*R*)-naproxen methyl ester ⇔ (*S*)-naproxen methyl ester → (*S*)-naproxen acid [1 + 1 = 2] **26**	ester racemization ester hydrolysis [two-phase system]	base lipase	100% ee/100% yield concept by combined bio−chemo catalytic action in a membrane reactor	40
(*R*)-(1-phenylethyl)amine ⇔ (*S*)-(1-phenylethyl)amine + AcOEt → (*S*)-*N*-(1-phenylethyl)acetamide + AcOH [1 + 1 = 2] **27**	racemization acylation [ethyl acetate]	palladium lipase	100% ee/100% yield oncept by combined bio−chemo catalytic action in one pot for enantiopure amines; enzymatic reaction in organic solvent, with 75% yield and 99% ee	41
(*S*)-1-phenylethanol ⇔ (*R*)-1-phenylethanol + 4-chlorophenyl (*or* vinyl) acetate → (*R*)-1-phenylethyl acetate + 4-chlorophenol (*or* acetaldehyde) [1 + 1 = 2] **28**	racemization esterification [*t*-BuOH, cyclohexane, *T* 20−70°C]	ruthenium or: rhodium lipase	100% ee/100% yield concept by combined bio−chemo catalytic action in one pot; enzymatic reaction in an organic solvent; high yields and ee's; reaction times vary from several to more than 100 h	42 43 44 45 46 47 48
(*S*)-Allylic alcohols ⇔ (*R*)-Allylic alcohols + AcOR → (*R*)-Allylic esters + AcOH [1 + 1 = 2] **29**	racemization esterification [organic solvent, *T* 30−100 °C]	ruthenium lipase	100% ee/100% yield concept by combined bio−chemo catalytic action in one pot; enzymatic reaction in an organic solvent; yields over 80% with high ee's	49
(*S*)-2-phenyl-3-acetoxycyclohexene ⇔ (*R*)-2-phenyl-3-acetoxycyclohexene + H_2O → (*R*)-2-phenyl-3-hydroxycyclohexene [1 + 1 = 2] **30**	racemization (by double bond migration) ester hydrolysis [organic solvent, *T* 30−100 °C]	palladium lipase	100% ee/100% yield concept by combined bio−chemo catalytic action in one pot; enzymic reaction in an organic solvent; yields over 80% with high ee's	43

Table 1. (Continued)

conversions number of transformations [chemo + bio = total]	reaction type and conditions	catalysts and/or reagents	additional comments	refs
		Bio−Chemo (Entries 22−47) (Continued)		
(*S*)-β-hydroxy compounds ⇔ (*R*)-β-hydroxy compounds + AcOR → (*R*)-β-acetoxy compounds + ROH [starting compounds are β-hydroxy esters, ethers, acetals, and nitriles] [1 + 1 = 2] **31**	racemization (redox) esterification [organic solvent, *T* 30−100 °C]	ruthenium lipase	100% ee/100% yield concept for synthesis of β-acetoxy compounds by combined bio−chemo catalytic action in one pot; enzymic reaction in an organic solvent; yields up to 92%, ee's of 99%	50 51 52
(*S*)-β-azido alcohol ⇔ (*R*)-β-azido alcohol + AcOR → (*R*)-β-azidoalkyl acetate + ROH [1 + 1 = 2] **32**	racemization (redox) esterification [organic solvent, *T* 30−100 °C]	ruthenium lipase	100% ee/100% yield concept by combined bio−chemo catalytic action in one pot; enzymatic reaction in an organic solvent; yields up to 85%, ee's up to 99%	53
R-CO-R′ + alcohol ⇔ ketone + (*S*)-R-CHOH-R′ + (*R*)-R-CHOH-R′ + AcOR″ → (*R*)-R-CHOAc-R′ + HOR″ [1 + 1 = 2] **33**	racemization (redox) acylation [alcohol as H-donor; organic solvent]	ruthenium lipase	100% ee/100% yield concept by combined bio−chemo catalytic action in one pot by Samsung Fine Chemicals; enzymatic reaction in an organic solvent	54
(*R*)-α-amino acid ester ⇔ (*S*)-α-amino acid ester + AcOR → (*S*)-α-*N*-acylamino ester + ROH [1 + 1 = 2] **34**	racemization esterification [−20 °C, 88% ee, 85% conversion]	arylaldehyde lipase	100% ee/100% yield concept by combined catalytic action in one pot, i.e. imine-induced racemization coupled with enzymatic esterification in organic solvents	21 22
2 e^- + 2 H^+ + O_2 → H_2O_2 coupled with: thioanisole + H_2O_2 → (*R*)-methylphenyl sulfoxide [1 + 1 = 2] **35**	electrochemical reduction enantioselective sulfoxidation [water, pH 4, *T* 20 °C]	cathode chloroperoxidase	electrochemical formation of hydrogen peroxide, in situ used for enzymatic oxidation; total TON over 1 million, over 90% yield at 20 mM thioanisole	55
epichlorohydrin + 2 HCN → 3-hydroxyglutaronitrile + 2 H_2O → NH_4^+ (*R*)-3-hydroxy-4-cyanobutyrate [1 + 1 = 2] **36**	nucleophilic substitution nitrile hydrolysis [water, ambient conditions]	base nitrilase	carried out on gram scale by Diversa; reduces traditional six-step to a three-step synthesis	56
phenols + O_2 → *o*-hydroquinones → *o*-quinones + *cis*-alkenes → trisubstituted bicyclooctaenediones [1 + 1 = 2] **37**	oxidation Diels−Alder reaction [chloroform]	tyrosinase	carried out for *p*-substituted phenols with a two-enzyme system expressed in *E. coli* and used as such; the Diels−Alder reaction occurs spontaneously	57
CO_2 + MVH^+ → $HCOO^-$ + MV^{2+} coupled with: MV^{2+} + donor-H_2 + $h\nu$ → MVH^+ + H^+ + donor [1 + 1 = 2] **38**	reduction cofactor regeneration [water, pH 7, *T* 30 °C, 0.3 M triethanolamine, visible light irradiation]	formate dehydrogenase [methyl viologen] zinc porphyrin [triethanolamine as H donor]	bio−chemo CO_2 fixation with an enzyme and a water-soluble zinc porphyrin as visible light photosensitizer gave 10% yield of formic acid (in 0.1 mM concentration) after 4 h irradiation	58
L-lysine → (*S*)-6-oxo-2-aminohexanoic acid → (*S*)-pipecolic acid [1 + 2 = 3] **39**	alkylamino → aldehyde imine cyclization imine reduction [water, ambient conditions]	aminotransferase acid−base catalysis reductase	one-pot oxidative enzymatic ring closure without using reagents other than oxygen, as developed by Mercian Corp.	59

Table 1. (Continued)

conversions number of transformations [chemo + bio = total]	reaction type and conditions	catalysts and/or reagents	additional comments	refs
		Bio−Chemo (Entries 22−47) (Continued)		
cephalosporin C + O_2 + methyl tetrazolyl acetate + 2-mercapto-5 -methylthiadiazole → cefazolin + α-keto acid [1 + 3 = 4] **40**	oxidation amide hydrolysis amine acylation thioether formation [water, pH = 8 → 6.5 *T* 25 → 4 → 65 °C]	amino acid oxidase glutaryl acylase penicillin G acylase acid base catalysis	three-step chemo-enzymatic *β*-lactam antibiotic synthesis without intermediate workup steps in 95% overall yield	60
D-glucose + O_2 → D-gluconic acid + H_2O_2 + HCl → HOCl + alkene → (*R*/*S*)-epoxide + HCl + H_2O → (*R*)-epoxide + (*S*)-glycol [1 + 3 = 4] **41**	oxidation hypochlorite formation epoxidation epoxide hydrolysis [two-phase system]	glucose oxidase chloroperoxidase metal porphyrin [trace of HCl] epoxide hydrolase	model cascade design for studying the principle of compartmentalization of chemocatalysts and enzymes by self-assembling of immobilized enzyme and catalyst in water	18
dirty laundry + H_2O + MO_x →→→→→→→→ dirt$(OH)_x$ + clean laundry [1 + 6 = 7] **42**	hydrolysis protein amide hydrolysis fat ester hydrolysis starch acetal hydrolysis cellulose acetal hydrolysis oxidation [water, pH 10, *T* 25−80 °C]	base proteases lipase amylase cellulase oxidase	modern detergent formulations have up to six different enzymes in them: an advanced multicatalytic one-pot conversion with the washing machine as the in-house catalytic reactor that simultaneously separates the product (clean laundry) from waste (dirt and detergent ingredients)	61
benzaldehyde + HCN ⇔ (*R*/*S*)-mandelonitrile + vinyl acetate → (*S*)-mandelonitrile acetate + acetaldehyde [2 + 1 = 3] **43**	cyanohydrin formation racemization acylation [organic solvent]	HO^- exchanger lipase	100% ee/100% yield concept by combined bio−chemo catalytic action in one pot; enzymatic reaction in organic solvent	62 63
aldehyde + HCN + NH_3 ⇔ (*R*)-α-aminonitrile + (*S*)-α-amino-nitrile + H_2O → (*S*)-α-amino acid + NH_3 [2 + 1 = 3] **44**	Strecker reaction racemization nitrile hydrolysis [water, pH 7, *T* 30 °C]	acid−base catalysis nitrilase	100% ee/100% yield concept by combined catalytic action in one pot, yielding an enantioselective Strecker synthesis of amino acids	64 65 66
aryl-CHO + HCN ⇔ (*S*)- and (*R*)-aryl-CH(OH)CN → (*R*)-aryl-CH(OH)COOH + NH_3 [2 + 1 = 3] **45**	cyanohydrin formation racemization nitrile hydrolysis [water:MeOH 10:1, pH 8, *T* 37 °C]	acid−base catalysis nitrilase	100% ee/100% yield concept by combined catalytic action in one pot for syntheses of mandelic acids, aryllactic acids, and their analogues on gram scale; high yields and ee's	67
D-galactose + O_2 → galactose dialdehyde + 2 butylamine→ 2 H_2O + galactose 1,6-di(butylimine) → 1,6-di(butylamino)- hexo-2,5-diulose [2 + 1 = 3] **46**	oxygenation imine formation Amadori rearrangement [water → methanol pH 7, *T* 10 → 50 °C]	galactose oxidase catalase acid−base catalysis oxalic acid	model reaction for enzymatic protein cross-linking, e.g. valorization of lactose-containing whey from cheese making. C-13 labeling used for in situ structure determination	68
D-galactoside + O_2 → 6-aldehydo-D-galactoside → H_2O + 3,4-dehydrated 6-aldehydo-D-glucoside + H_2 → 6-aldehydo-4-deoxy- D-glucoside + $NaBH_4$ → 4-deoxy-D-glucoside and: reductive amination and oxidation reactions toward amino sugars and sugar acid derivatives, respectively [>3 + 1 = >4] **47**	oxygenation dehydration hydrogenation reduction reductive amination oxidation [water, pH 7 *T* 10 → 70 → 25 °C]	galactose oxidase catalase L-proline palladium platinum [ClO_2^-]	quantitative one-pot cascade involving an enzyme, a homogeneous and a heterogeneous catalyst, followed by aqueous chemical conversions leads to 90% reduction of reagents and an operating time of hours instead of days as compared to protective group step-by-step synthesis	69

Table 1. (Continued)

conversions number of transformations [chemo + bio = total]	reaction type and conditions	catalysts and/or reagents	additional comments	refs
		Chemo−Chemo (Entries 48−62)		
(*R*)-α-benzamido-*β*-keto ester ⇔ (*S*)-α-benzamido-*β*-keto ester + H_2 → (*S*,*S*)- α-benzamido-*β*-hydroxy ester [2 + 0 = 2] **48**	racemization hydrogenation [organic solvent]	acid−base catalysis ruthenium [chiral ligands: 4,4′-bis(1,3-benzodioxole)-5,5′-diyl-bis(diaryl-phosphine)s]	100% de+ee/100% yield concept by combined chemo−chemo catalysis, as developed by Takasago Int. Corp.; *S*,*S* versus *R*,*R* product formation dependent on chirality of the catalyst ligand; high de/ee's (>98%)	70
R-CHO + Ph-NH_2 → R-CH═HH-Ph + Ph-C≡C-H → R-CH(NH-Ph)-C≡C-Ph [2 + 0 = 2] **49**	imine formation nucleophilic addition [water or neat, *T* 40 °C]	ruthenium copper	simultaneous C−H activation of the alkyne by Ru and the in situ formed imine by Cu; reactions are carried out in water in open air; yields up to 95%, ee's up to 93%	71
alkene + *N*-methylmorpholine-*N*-oxide → glycol + *N*-methylmorpholine coupled with: *N*-methylmorpholine + H_2O_2 → *N*-methylmorpholine-*N*-oxide [2 + 0 = 2] **50**	dihydroxylation cofactor regeneration [acetone/water mixture]	homogeneous OsO_4 biomimetic flavin or: heterogeneous OsO_4 on titanium/MCM-41	clean alkene to glycol conversion using oxygen or hydrogen peroxide as oxidative reagent with in situ regeneration of the morpholine oxidant; glycol yields up to 98%	72 73
alcohols + acceptor → carbonyl compounds + acceptor-H_2, coupled with: acceptor-H_2 + O_2 → acceptor + H_2O [2 + 0 = 2] **51**	alcohol dehydrogenatio cofactor regeneration [chlorobenzene, *T* 100 °C, 10 atm]	quinones or: ruthenium [TEMPO]	bio-inspired catalytic oxidation of alcohols through in situ artificial cofactor regeneration with yields up to 99%	74 75 76 77 78
lignin-H + cellulose + $[V^{5+}AlW_{11}O_{40}]^{6-}$ → lignin-$O_{x\,(soluble)}$ + cellulose + H^+ $[V^{4+}AlW_{11}O_{40}]^{7-}$ + O_2 → CO_2 + H_2O + cellulose + $[V^{4+}AlW_{11}O_{40}]^{6-}$ [2 + 0 = 2] **52**	selective oxidation catalyst regeneration [water, ambient conditions]	$[V^{5+}AlW_{11}O_{40}]^{6-}$ $[Al_3W_{10}O_{38}]^{7-}$ $[Al_2W_{11}O_{39}]^{6-}$ $[V_2W_4O_{19}]^{4-}$ $[W_7O_{24}]^{7-}$	removal of lignine from wood pulp by oxidative solubilization of lignine (leaving cellulose intact), followed by further complete oxidation; an ingenious cooperation of various heteropolytungstate anions in water, using O_2 as the sole oxidant	79
erythro-CHOH-CHOH-CHO → *erythro*-CHOH-CHOH-CH═CH-COOR → *galacto*-CHOH-CHOH-CHOH-CHOH-COOR [2 + 0 = 2] **53**	Wittig reaction dihydroxylation	[Ph_3P═CHCOOR] OsO_4 [NMO]	one-pot chemo diastereoselective −CHOH−CHOH− chain extension of sugars without the need for protective groups	80
pinacol → pinacolone + H_2O + malonitrile → 1,1-dicyano-2-*tert*-butyl -1-butene [2 + 0 = 2] **54**	pinakol rearrangement condensation reaction [benzene, reflux]	polymer acid polymer base	demonstration of simultaneous acid and base catalysis in one pot	81
Heck coupling reaction + oxidation by *N*-methylmorpholine-*N*-oxide → glycols, coupled with: *N*-methylmorpholine + H_2O_2 → *N*-methylmorpholine-*N*-oxide + H_2O [3 + 0 = 3] **55**	C−C bond formation dihydroxylation cofactor regeneration [8 h 70 °C, 12 h 25 °C]	palladium(II) tungsten(VI) osmium(VI)	full catalytic approach by use of trifunctional heterogeneous catalyst of transition metals in a single layered double hydroxide matrix with in situ regeneration of the morpholine oxidant; yields up to 95% with ee's up to 99%	82
(*R*)-amine + racemic ketone ⇔ 4 isomeric imines → 1 crystalline imine + H_2O → (*R*)-ketone + (*R*)-amine [3 + 0 = 3] **56**	imine formation (reversible) selective crystallization hydrolysis	acid−base catalysis [(*R*)-amine]	coupled selective crystallization of dynamic equilibrium of imines, for the one-pot production of enantiopure ketones as developed by Eli Lilly	83

Table 1. (Continued)

conversions number of transformations [chemo + bio = total]	reaction type and conditions	catalysts and/or reagents	additional comments	refs
	Chemo−Chemo (Entries 48−62) (Continued)			
HO-[anthracene]-OH + O_2 → O=[anthracene]=O + H_2O_2 + propene → propene oxide + H_2O coupled with: O=[anthracene] = O + H_2 → HO-[anthracene]-OH [3 + 0 = 3] **57**	hydrogen peroxide formation epoxidation cofactor regeneration (separate hydrogenation step) [organic solvent, *T* 65 °C, 6 h]	anthraquinone $[PO_4(WO_3)_4]^-$ $[R_4N]^+$ metal hydrogenation catalyst	direct epoxidation with in situ generated H_2O_2 that dissolves the tungsten-containing catalyst; the soluble active tungsten species catalyzes the epoxidation; as H_2O_2 is used up, the catalyst becomes insoluble again, simplifying its recovery and allowing simple rehydrogenation of the anthraquinone formed; yields of 85%	84
Pol−O-CO-CH_2CN + MeCHO → Pol−O-CO-CH(CN)=CHMe + MeOOC-CH_2-COOMe → Pol−O-CO-CH(CN)- CHMe-$CH(COOMe)_2$ + CH_2=CHCOMe → Pol−O-CO-C(CN)[CHMe-CHMe- $CH(COOMe)_2$]-CH_2CH_2COMe + MeOH → Pol−O−H + MeO-CO-C(CN)[CHMe-CHMe- $CH(COOMe)_2$]-CH_2CH_2COMe [4 + 0 = 4] **58**	aldol reaction Michael reactions transesterification [THF, *T* 60 °C]	$RuH_2(PPh_3)_4$	four-component reaction involving sequential aldol-Michael−Michael reactions by C−H activation by the ruthenium catalyst take place with 90% diasteroselectively; overall yield 40%, after transesterification for cleavage off the polymeric support	85
R-CH_2-COCl → R-CH=C=O + HCl together with: R′OOC-CH=O + H_2N-R″ → R′OOC-CH=N-R″ + H_2O followed by: R-CH=C=O + R′OOC-CH=N-R″ → R-CH-C=O |	+ H_2O → amino acids R′-CH-N-R″ [4 + 0 = 4] **59**	ketene formation imine formation [2 + 2] addition hydrolysis	Na_2CO_3 (optional) Lewis acid (optional) Pol−benzoylquinine	substrates in solution are converted to products by flowing through columns containing solid-phase-bound reagents, catalysts, and scavengers that pick up byproducts; the chiral alkaloid (benzoylquinine) can catalyze all four reactions; the Lewis acid increases the optical yield by more than 40%; the column could be used >100 times	86
amino acids →→→→ tripeptide [<5 + 0 =<5] **60**	peptide synthesis [water, ambient conditions]	DNA templates	novel use of DNA templates to direct multistep syntheses; linker and purification strategies or separating reagent DNA from products	87
C_6H_{13}CHO + $C_4H_9NH_2$ +H_2CO + $C_6H_5CH_2$CH(CN)-CO-N$(CH_2CH_2)_2$O + C_6H_5C≡CH−COO−C_6F_5 + *N*-phenylmaleinimide →→→→→→ hexasubstituted benzene [5 + 0 = 5] **61**	condensation reactions oxazole formation cycloaddition reactions [toluene, *T* 25 → 60 → 0 → 25 → 110 °C]	camphorsulfonic acid	the three-component synthesis of an oxazole is compatible with two subsequent cycloaddition reactions, allowing the assembly of complex molecules in one pot with overall yields of 32−67%	88
H_2CO + $BuNH_2$ + HPF_6 + glyoxal →→→→→ 1,3-dibutylimidazolium PF_6^- [5 + 0 = 5] **62**	imine formation cyclocondensation [neat, *T* 25 °C]	acid−base catalysis	highly efficient four-component one-pot synthesis of water-immiscible ionic liquids by simply mixing the four reagents	89

From the examples on laboratory scale (entries **1−62**) and on pilot or industrial scale (entries **63−73**) the following major features are apparent for the three different types of cascade transformations:

(1) By far, most bio-bio examples have been reported from the 1970s in the field of carbohydrates using combinations of enzymatic conversions (e.g. the many one-potmultistep examples given in entries **18** and **19**) as well as for the in situ cofactor regeneration of enzymatic redox reactions towards amino and hydroxy acids (e.g. entries **1−3**);

(2) After some early examples of bio−chemo combinations in the 1980s (e.g., entry **22**), there has been more than a decade of "silence", followed by a clearly increasing

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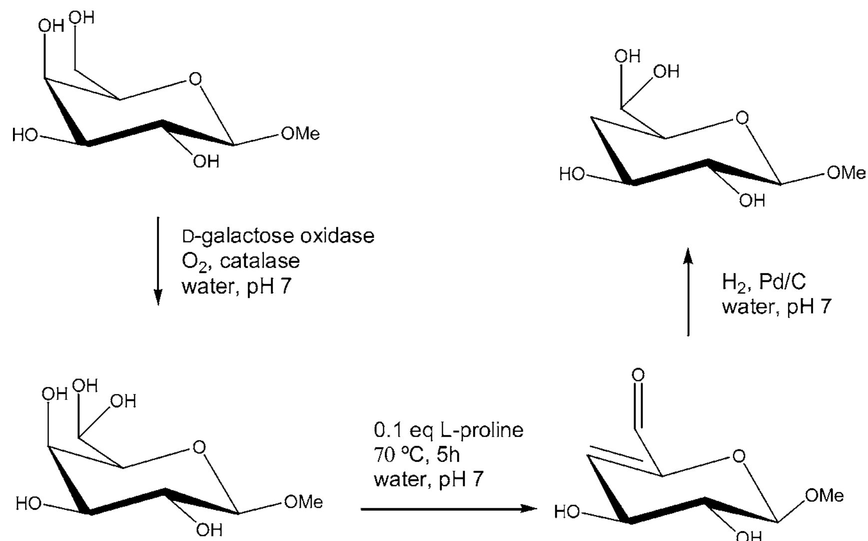

Figure 4. **Cascade three-step catalytic conversion in one pot in water, using both bio- and chemocatalysts: quantitative, clean, and rapid method as compared to traditional organic synthesis (entry 47).**

interest from the mid 1990s in the field of dynamic kinetic resolution processes (e.g. the chemocatalysed racemization combined with enzymatic conversion as given by entries **25**−**34** and **65**);

(3) Up to the late 1990s, combined multistep chemo−chemo conversions are restricted to a few catalytic examples,

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i.e., there has been not much effort or interest to develop a toolkit of chemocatalytic reactions that are mutually compatible with respect to reaction conditions. Therefore, chemocatalysts have not yet reached the same level of mutual compatibility as have biocatalysts. Some recent examples prove, however, the potential power of chemo−chemo catalytic cascades (e.g., entries **61** and **71**).

The unadvanced state of cascade syntheses can also be seen from the number of separate steps making up a cascade. More than 50% of the examples are two-step reactions of which are numerous coupled redox reactions, and only 25% consist of four steps or more. From the number of publications over the years it appears that the development of one-pot procedures grows steadily (Figure 5). As most of the enzymes function under compatible ambient conditions, bio−bio cascades have already been successfully developed some decades ago, including in situ cofactor regenerations, whereas most of the bio−chemo and chemo−chemo (catalytic) cascades have been coming up during the past 5 years (Figure 6). Relevant types of combined bio−bio-, bio−chemo-, and chemo−chemocatalyzed reactions are discussed below, respectively.

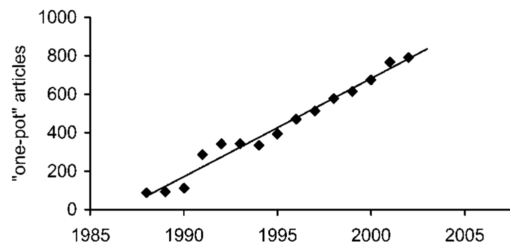

Figure 5. **Steadily increasing interest in one-pot cascade-type of procedures over the years since the mid 1980s.**

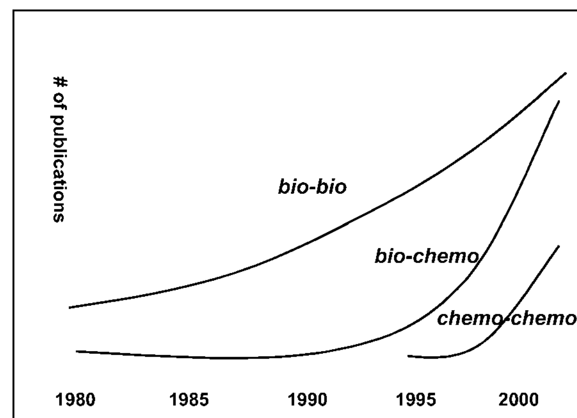

Figure 6. **Relative trends of the various types of cascade conversions over the years.**

Bio−Bio Catalytic Cascade Conversions (Entries 1−21 and 63−64). As enzymes easily may work together under comparable reaction conditions, combined enzymatic reactions are by far the most early and abundantly studied. Apart from various two-enzyme conversions including in situ redox cofactor regenerations (entries **1**−**5**, **7**, **8**, **13**, **14**, **63**, and **64**), multienzyme carbohydrate conversions on multigram scale have been accomplished successfully using up to seven different enzymes in one pot (entries **15**−**20**). The record until now is the 12-step enzymatic conversion in one pot, although on a milligram scale, of the corrin moiety of vitamin B_{12} (entry **21**). Another interesting case is the one-pot four-enzyme cascade conversion of glycerol into a heptose sugar on gram scale, in which a pH switch method is applied to temporarily turn off one of the enzymes involved (entry **15** and Figure 7).

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Table 2. **Cascade conversions from kilogram pilot plant to multiton industrial scale**

conversions number of transformations chemo + bio = total]	reaction type and conditions	catalysts and/or reagents	additional comments	refs
	Bio−Bio (Entries 63−64)			
α-keto acids + NH_3 + NAD(P)H → L-α-amino acids + NAD(P) coupled with: formate + NAD→ NADH + CO_2 [0 + 2 = 2] **63**	reductive amination; cofactor regeneration [water, pH 8, *T* 25 °C]	amino acid dehydrogenase; formate dehydrogenase	multikilogram scale, by Degussa, in a membrane reactor (also in a continuous mode)	6 90
D-hydantoins ⇔ L-hydantoins →→ L-amino acids [0 + 3 = 3] **64**	racemization hydrolysis [water, pH 8.5, *T* 50 °C]	racemase L-hydantoinase carbamoylase	multi-100-kg scale, e.g. L-methionine, L-norleucine, L-2-aminobutyric acid, L-3-(3′-pyridyl)alanine, by Degussa	70
	Bio−Chemo (Entries 65−67)			
(*S*)-sec-alcohols ⇔ (*R*)-sec-alcohols + RCOOR′ → (*R*)-sec-alkyl esters + HOR′ [1 + 1 = 2] **65**	racemization; transesterification [organic solvent]	ruthenium lipase	100% ee/100% yield concept using one-pot metal catalyzed racemization coupled with enzymatic enantioselective esterification in organic solvents; multiton-per year scale, by DSM, for enantiopure alcohol production	70
Z-L-Asp-OH + DL-H-Phe-OMe → *Z*-L-Asp-L-Phe-OMe + D-H-Phe-OMe → 1:1 mixed precipitate → aspartame [1 + 1 = 2] **66**	peptide coupling selective crystallization [water]	thermolysin	multiton precipitation driven peptide synthesis of aspartame by Holland Sweetener Company	91
L-hydantoins ⇔ D-hydantoins →→ D-amino acids [1 + 2 = 3] **67**	racemization hydrolysis [water, pH 8.5, *T* 50 °C]	base D-hydantoinase carbamoylase	multiton scale, e.g. of D-*p*-hydroxy-phenylglycine, by Kanega and Recordati	19 92
	Chemo−Chemo (Entries 68−73)			
benzaldehyde + 4-methoxyaniline → *N*-(4-methoxyphenyl)benzalimine + [in situ $AcOCH_2COCl$ → AcOCH=C=O *transformation*] → *N*-(4-methoxyphenyl)-α-acetoxy-*β*-phenyl-*β*-lactam [3 + 0 = 3] **68**	imine formation [2+2] cycloaddition toluene (water removal by reflux) heptane water	tertiary amine	straightforward large-scale three-step preparation of Taxol intermediate, by Bristol-Myer, without isolation of intermediates	89
2-phenyl-1,3-propanediol + Me_3SiCl → Ph-CH(CH_2OH)-CH_2O−Si Me_3 + 1,1′-carbonyldiimidazole + NH_3 → Ph-CH(CH_2OH)-CH_2O−$CONH_2$ [3 + 0 = 3] **69**	monosilylation; esterification aminolysis THF; *T* 25 → −78 °C]	[NaH]	most efficient method for monocarbamate felbamate metabolite synthesis in 90% overall yield; high reagent and energy use	93
ascomycin →→→ ascomycin derivative SDZ ASD 732 [showing bioactivity for allergic treatment of contact dermatitis] [3 + 0 = 3] **70**	epimerization isomerization rearrangement [MeCN + Et_3N; *T* 74 °C; 12 h]	KOH	pilot plant synthesis of semisynthetic ascomycin by Novartis; overall yield of 53%, after chromatography and crystallization, with 95% purity	94

Table 2. (Continued)

conversions number of transformations chemo + bio = total]	reaction type and conditions	catalysts and/or reagents	additional comments	refs
	Chemo−Chemo (Entries 68−73) (Continued)			
R-CO-R′ + $MeNH_2$ → R-C=NMe-R′ + H_2 → *cis/trans* R-CHNHMe-R′ mixture + D-mandelic acid → *cis*-R-CHNHMe-R′ D-mandelate + HCl → R−CHNHMe-R′ HCl salt [R-CO-R′ stands for 4-(3,4-dichlorophenyl)aryl-1-tetralone] [4 + 0 = 4] **71**	imine formation reductive amination cis/trans separation [ethanol; ethyl acetate; toluene; H_2; methylamine; D-mandelic acid]	acid base catalysis $Pd/CaCO_3$ [D-mandelic acid]	cascade-type of optimization of traditional multistep approach by using less different solvents and recovery operations results in much less waste and 20% higher yield; full-scale operation, by Pfizer, for sertraline synthesis	89 95
alanine + Ac_2O → Me-CH(NHAc)-COMe +NC-CH_2-CN → 1-amino-2-cyano-4,5-dimethyl-pyrrole + HC(OEt_3) + 3-chloroaniline →→ XXX4-(3-chlorophenylamino)−5,6-dimethyl- 7*H*-pyrrolo[2,3-*d*]pyrimidine [4 + 0 = 4] **72**	Dakin−West reaction pyrrole condensation pyrimidine condensation Dimroth rearrangement [Ac_2O + Et_3N → water → EtOH → glycol; *T* 50 → 95 °C; 30 h overall]	4-(dimethylamino)- pyridine [NaOH]	pilot plant procedure in 60% overall yield by Novartis without any need of chromatography, extraction or waste treatment; evaporation steps required for the solvent changes in the cascade	96
oligonucleotide synthesis on a solid support using phosphoramidite reagents [20 + 0 = 20] **73**	phosphate esterification [packed bed reactor, full synthesis cycle less than 5 h]	pol-reagents	scaling-up multi-mg to multi-100 g syntheses for clinical trials by Isis Pharmaceuticals, in 50% overall yield, using tightly packed 35-cm-diameter tubes; solvent reduction and excess of reactants of 1.7 instead 10−14-fold; cost reduction of 99%	97

Very recently, an impressive one-pot six-steps enzymatic synthesis of riboflavin from glucose has been reported in an overall yield of 35−50% (entry **17**). Six different enzymes are involved in the various synthesis steps, while two other enzymes take care of the in situ cofactor regenerations. This example again shows that many more multienzyme cascade conversions will be developed soon, as a much greater variety of enzymes in sufficient amounts for organic synthetic purposes will become available through fast developments in genomics and proteomics. In a way, modern detergent formulations containing up to six different enzymes in them is an industrial example of an advanced multicatalytic one-pot conversion with the washing machine as the in-house catalytic reactor (entry **42**).

Bio−Chemocatalytic Cascade Conversions (Entries 22−47 And 65−67). A very first example involving the combined action of an enzyme and a metal catalyst is the direct one-pot conversion of glucose into mannitol, which is 3-fold more expensive than glucitol, the sole product from common glucose hydrogenation (entry **22** and Figure 8). Here, the isomerase enzyme converts glucose into a ∼1:1 glucose−fructose mixture and takes care that this mixture remains in equilibrium, while at the same time the copper catalyst hydrogenates preferentially fructose from this equilibrium into mannitol. The high temperature (70 °C) and hydrogen pressure (70 atm) had no negative influence on the enzyme's activity or stability.

This principle of a dynamic equilibrium between two compounds by one catalyst in combination with a selective conversion of one of those by a second catalyst, is of great importance for the so-called 100% ee/100% yield synthesis of enantiomerically pure compounds from racemic starting materials. A number of recent examples of such dynamic kinetic resolutions on lab-scale have been reported (entries **25**−**34** and **43**−**45**) using the concomitant action of a chemocatalyst and a biocatalyst (Figure 9). Without such a combination of two catalysts in one reactor, a maximum yield of either only 50% can be obtained or separate recovery and racemization steps are required.

In particular, the combined action of a transition-metal catalyst and a lipase in organic solvents for the racemization and esterification steps, respectively, have been applied for the conversion of racemic secondary alcohols into their esters in high yield and high optical purity. The attractiveness of this type of syntheses for enantiomerically pure compounds from relatively inexpensive racemates has already found its way into the fine chemical industry. As a first example, an industrial multiton per year production will start this year

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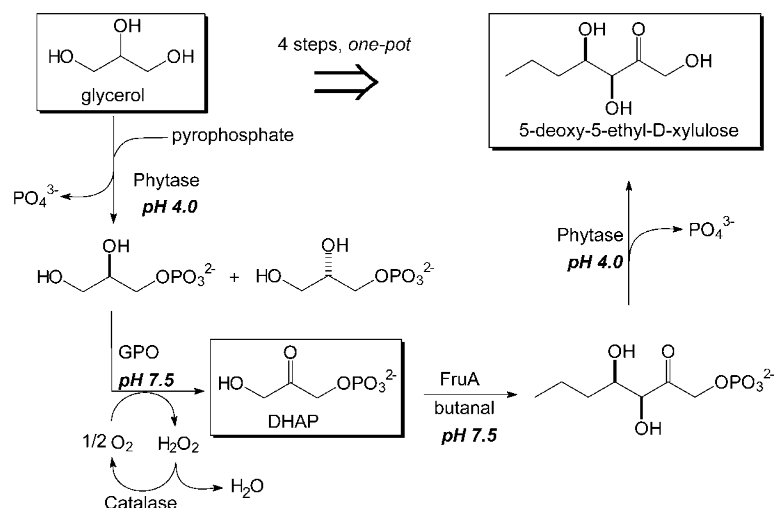

Figure 7. **Multienzyme one-pot example: cascade conversion of glycerol into a heptose sugar through consecutive phosphorylation, oxidation, aldol reaction, and dephosphorylation (entry 15).**

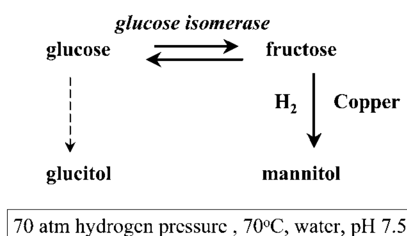

Figure 8. **Early example of combined enzyme and metal catalysis: the one-pot conversion of glucose to mannitol (entry 22).**

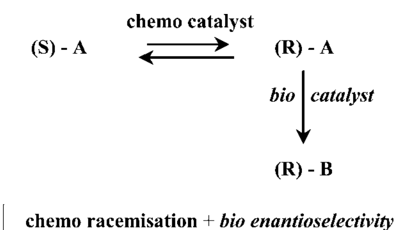

Figure 9. **Complete conversion of a racemate A into an optically pure compound B by the cooperation of two catalysts in one pot (entries 27−33 and 65).**

by DSM Fine Chemicals using such a one-pot procedure in an organic solvent. Only one of the enantiomers of the racemic secondary alcohol is esterified by the enzyme, while the homogeneous metal catalyst racemizes both enantiomers of the alcohol. In this way, a nearly quantitative yield of enantiopure ester is obtained (entry **65**).

Additional three- or more-steps bio−chemo cascades are still rare (entries **39**−**41** and **47**, cf. Figure 4), due to the incompatibility of many chemocatalytic with enzymatic conversions in terms of reagents, solvent, pH and/or temperature. To overcome such incompatibilities a self-assembling multicomponent system, consisting of three enzymes and a porphyrin catalyst, has been designed on the basis of amphiphilic polymer−enzyme bio-hybrids (entry **41**).

Chemo−Chemo (Catalytic) Cascade Conversions (Entries 48−62 and 68−73). As mentioned above, combined catalytic conversions through combinations of chemocatalytic conversions are not well represented. Although the organic chemist has the disposal of an extensive synthetic and catalytic toolkit, the large differences in reaction conditions often hinder the combined use of those tools in one-pot conversions or in a cascade mode without recovery steps. On the other hand, an increasing academic interest and success has been demonstrated recently by a number of multistep syntheses without isolation of intermediate products (e.g., entries **58**−**62**). In particular, the recent in situ acid-catalyzed three-component synthesis of an oxazole that is compatible with two subsequent cycloaddition reactions is impressive. This one-pot procedure is simple to operate, gives good yields of complicated ring structures, and is also highly adaptable to diversity-oriented parallel syntheses (entry **61**).

On an industrial scale, three chemical conversion steps have been combined without isolating intermediates by replacement of four different solvents by just one in the synthesis of the drug sertraline by Pfizer (entry **71**). The changes reduced the solvent requirement from 60 000 to 6000 gal per ton of sertraline and eliminated the use of 440 metric tons of titanium dioxide, 150 metric tons of 35% hydrochloric acid, and 100 metric tons of 50% sodium hydroxide per year, reducing the environmental burden and saving hundreds of thousands of dollars.

Apparently, there has been too little focus thus far to develop a toolkit of chemocatalytic conversions that are mutually compatible (great differences in solvent, temperature, sensitivity for air and moisture, reactants, e.g., entries **69** and **72**), as is the case for enzymatic reactions in Nature. In fact, this confirms the main difference in approach between organic synthesis and biosynthesis: organic synthesis employing a maximum diversity in reagents and conditions versus biosynthesis exploiting subtlety and se-

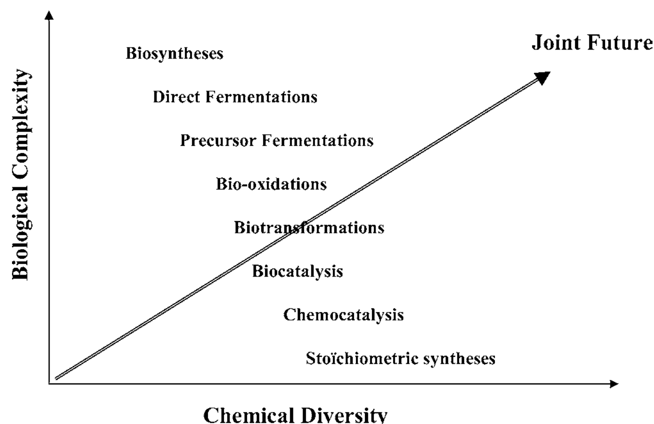

Figure 10. **Synthesis from chemical and biological perspective (after J. M. Lehn).**

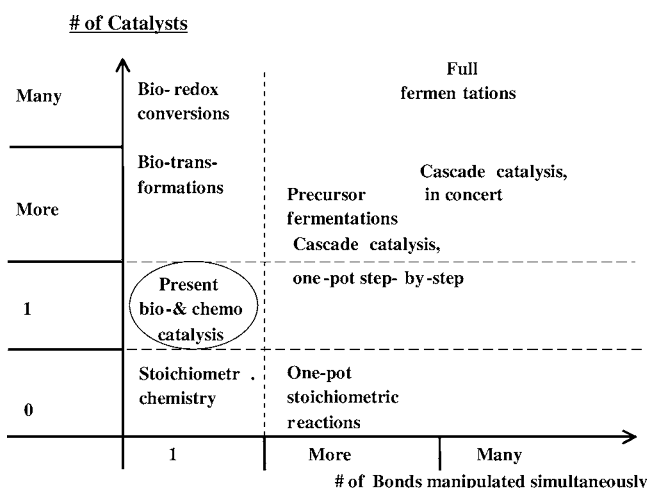

Figure 11. **Shift of synthesis methodology: multibonds manipulation by multicatalytic transformations.**

lectivity from a small range of materials and conditions (Figure 10).

As many starting materials and products of the fine chemical industry require an organic solvent instead of water, combined conversions in one or two "standard" organic solvents offers a powerful methodology that yet has to be exploited. This, of course, requires redeveloping part of the synthetic toolkit of organic chemistry to perform multistep organic syntheses in a combined catalytic mode. At the same time a lot of new synthesis and catalysis methodologies need to be developed to fill the full spectrum of possibilities for molecular assembly. On the long term, most of the present-day stoichiometric chemistry as well as bio- and chemocatalytic conversions in multistep syntheses will be gradually replaced by cascade catalysis in concert and full fermentations, or combinations thereof (Figure 11).

Studying Combined Catalytic Reactions. Investigations of combined catalytic conversions without isolation of intermediate products require appropriate in situ analytical methods to know what is really happening during the consecutive conversions. Quite a powerful window to this information is the use of selective isotope (e.g., ^{13}C, ^{15}N, ^{17}O)-enriched starting materials in combination with NMR.[98] In this way, a sequence of conversions can be well characterized, even in situ in complicated matrixes of catalysts, reagents, and mixed solvent systems, as applied to the three-catalytic one-pot reaction mentioned above (Figure 4, entry **47**) as well as in our structural investigations of chemo-enzymatic cross-linking phenomena of carbohydrate protein mixtures (entry **46**). In addition, further optimisation requires the application of factorial design and high throughput experimentation, as cascade processes involve a large number of mutually dependent kinetic parameters and molecular interactions.

Appropriate Technologies for Cascade Conversions, Including in Situ Product Recovery. Successful development and practical implementation of cascade catalysis and multistep conversions in concert requires a multidisciplinary approach, ranging from genetic engineering to reactor design. In particular, novel combinations of new reactor concepts, catalytic materials, and separation techniques will offer the synergy to afford multistep sustainable synthesis methodologies that are reminiscent of what occurs in the living cell. Apart from the requisite of very high selectivity for each transformation in cascade conversions, the in situ removal of byproducts and spent reagents has to be further developed by integrated process design. Therefore, a number of relevant recent technological trends in the fields of reactor design, compartmentalization, medium engineering, new catalytic and synthetic methods, and cell factory design have been summarized below.

Reactor Design. Porous ceramic bricks known as monoliths, widely used in automotive catalysis, can serve jointly as catalyst support and chemical reactors. Compared with packed-bed reactors, monoliths can be more efficient by a factor of 100 as they are a collection of parallel microreactors. It provides a way of applying the microreactor concept to an industrial setting and is broadly applicable, e.g., hydrogenation and oxidation, enzymatic reactions, enzymatic reactions, and organic synthesis.[99,100]

Membranes and structured multiphase reactors are means to achieve a compartmentalization of catalysts and reaction media, e.g. structured multiphase minireactors for multistep cascade conversions through the combined use of enzymes and transition-metal catalysts.[101] The advantages of a so-called structured reactor, instead of the traditionally applied stirred batch reactor, are the following: compactness, energy-efficiency, high selectivity, lower amounts of solvent, and easy scale-up.[100] For instance, enzymatic bioreactors with both high-flow characteristics and mechanical stability on macroporous monoliths have been designed. Covalent immobilization of the enzyme is achieved in a single reaction step using the azlactone functional groups of the monolith. High catalytic activity of the monolithic reactor is maintained even at a flow velocity of 180 cm/min, which substantially exceeds those reported for packed bed reactors, for the

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hydrolysis of both low- and high-molecular weight substrates such as L-*N*-benzoylarginine ethyl ester and casein by immobilized trypsin.[102]

There is a huge potential in the combination of biocatalysis and electrochemistry through reaction engineering as the linker. An example is a continuous electrochemical enzyme membrane reactor, which showed a total turnover number of 260 000 for the enantioselective peroxidase-catalyzed oxidation of a thioether into its sulfone by in situ cathodic generated hydrogen peroxide, much higher than achieved by conventional methods (entry **35**). The in situ titration circumvented fast deactivation of the peroxidase enzyme and allowed, for the first time, the application of chloroperoxidase in a continuously operated reactor system with low enzyme consumption.[55]

Compartmentalization. Membrame-enclosed enzymatic catalysis (MEEC) has been developed as a useful, practical new method for the manipulation of enzymes in organic synthesis. The enzyme in soluble form is enclosed in commercially available dialysis membranes. It combines the simplicity of use of soluble enzymes with certain of the advantages of immobilized enzymes. Containment permits separation of the enzyme from the reaction medium, straightforward separation of the product, and recovery of the enzyme for reuse.[103]

Two types of continuous membrane reactors have been applied for oligomer- or polymer-bound homogeneous catalytic conversions and recycling of the catalysts. In the so-called dead-end-filtration reactor the catalyst is compartmentalized in the reactor and is retained by the horizontally situated nanofiltration membrane. Reactants are continuously pumped into the reactor, whereas products and unreacted materials cross the membrane for further processing. However, undesired accumulation of the catalyst near the membrane can occur. Using a loop reactor, such behaviour is prevented, since the solution is continuously circulated from the reactor through the vertically situated membrane unit.[104]

Column chemistry and catalysis is applied to execute a synthetic sequence and deliver a pure product without separate workup, purification, and catalyst recovery steps. Substrates in solution are converted to products by flowing through solid-phase-bound reagents and catalysts.[105]

Template-synthesized nanotubular membranes are enabling new approaches to separations and conversions. Enzymes can be trapped within a nanotubular membrane by capping both faces of the membrane with a thin layer of porous polymer. In effect, arrays of enzyme-filled microcapsules are formed. Small molecules pass through the porous polymer plugs, but not the enzyme, which float freely in the confined space. Loaded with enzyme, the membrane works such as a bioreactor.[106]

Medium Engineering. Catalytic methods that use ecologically benign reaction media and innovative separation schemes are key to future developments. In particular, if the alternative media are sufficiently different from organic solvents to exploit some of their specific features for reaction tuning, or even new chemistry. Carbon dioxide and ionic liquids are at the extremes of the polarity and volatility scales.[107,108]

Fluorocarbon solvents can be omitted from biphasic catalysis reactions using the temperature-dependent solubility in octane of the solid phosphine $P[CH_2CH_2 (CF_2)_7CF_3]_3$. The catalyst is insoluble in octane at room temperature, but upon heating it becomes soluble, and the reaction proceeds. After cooling, the catalyst precipitates out of the reaction mixture and is recovered by decanting the liquid. In the same manner, reactions also could be carried out in a mixture of the neat reactants, i.e. without the need of any solvent.[109] Alternatively, the same principle can be applied by using the fluorinated boronic acid 3,5-bis$[(CF_2)_9CF_3]_2$ in *o*-xylene.[110]

New alternatives to bead syntheses are developed by the use of so-called precipitons that ease separation of products from solution-based reaction mixtures, e.g. *cis*-stilbene-based precipitons that become insoluble in organic solvents upon *cis*-to-*trans* isomerization, poly(ethylene glycol)s that can be precipitated out by adding other solvents, polyfluorinated reagents that are separated by partitioning into fluorous solvents, organic tags capable of chelating a metal that can be recovered by resin-bound metals.[111]

Precipitation-driven synthesis offers the possibility to obtain high reaction yields using very low-volume reactors and is finding increasing applications in biocatalysis. Straightforward prediction of when such a precipitation-driven reaction will be thermodynamically feasible is possible as demonstrated for a range of enzyme-catalyzed peptide syntheses. The methodology is quite general and is therefore expected to be applicable to a wide range of other (bio)-catalyzed reactions.[91,112]

A bottom-up strategy is to develop cost-effective green processes by gathering as much physicochemical data about the components of the reaction as possible and using the data to define reaction conditions that can also be used for workup and isolation. Key to this strategy is the availability of physicochemical data, such as solubility and crystallization characteristics of reactants, products, and byproducts. For example, by changing the reaction solvents in Bristol-Myer Squibb's synthesis route of the anti-cancer drug Taxol, the product crystallized out of solution and could be isolated by simple filtration rather than by extraction. With fewer unit operations, the yield is much higher, and by cutting down the number of solvents from five to three, the process is also more environmentally friendly.[113]

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Conventionally, organometallic chemistry and transition-metal catalysis are carried out under an inert gas atmosphere, and the exclusion of moisture has been essential. On the other hand, the catalytic actions of transition metals under ambient conditions of air and water have played a key role in various enzymatic reactions, which is in sharp contrast to most transition-metal-catalyzed reactions commonly used in the laboratory. Quasi-Nature catalysis has now been developed using late transition metals in air and water, e.g. copper-, palladium-, and rhodium-catalyzed C—C bond formations, and ruthenium-catalyzed olefin isomerizations and C—H activations. Even a Grignard-type of reaction could be realized in water using a bimetallic ruthenium—indium catalytic system, with one to activate the carbonyl function and the other to catalyze the overall reaction. The air- and moisture-tolerable feature of the catalysis offers convenience in chemical synthesis involving small-scale combinatorial synthesis, large-scale manufacturing, catalyst recycling and in combinations with enzymatic conversions.[71]

New Catalytic and Synthetic Methods. Transformations using biocatalysts are now recognized as attractive alternatives for the synthesis of a number of important and commercially significant compounds that are complementary to the traditional chemical approaches. With the rapid access to unique enzymes having improved or novel properties from various sources of biodiversity, and from ingenious DNA recombination techniques along an evolutionary approach, it can be anticipated that critically shorter process development cycles will be achievable—in fact, this may be a precondition to overcome a persisting skepticism about biotechnology and biocatalysis that has limited thus far the full realization of its potential in the chemical industries. The fast and timely availability of effective new biocatalytic tools is required to tackle the current and future challenges presented by the demands of organic synthesis, in a sustainable manner.[114]

To access the most diversified range of enzymes that can be found in Nature, large genomic libraries are created by extracting DNA directly from environmental samples that have been collected from varying global habitats. A variety of methods have been established to identify novel activities through screening these libraries. For instance, this gave access to over 200 new nitrilases by overexpression as lyophilized cell lysates, used for evaluation of their particular biocatalytic functions.[67]

Next to full deployment of enzymes an increasingly important future challenge for synthetic chemists rests in the discovery and invention of new reagents, catalysts, and transformations coupled with innovative strategies that furnish increasingly complicated molecular structures (i.e., new drugs) from readily available starting materials with minimal cost and waste. This task is by no means easy, because it inevitably prescribes the development of selective, high-yielding reaction chemistry that proceeds at ambient temperature and pressure. Synthetic chemists have long excelled at the design of complex reagents, procedures, and conditions for a multitude of synthetic elaborations. When all else fails, protective groups or multistep sequences come to the rescue. However, we must do better, and the only way to improve is by discovering and identifying new reagents and catalysts, thus making previously unimagined transformations possible. A marriage of inorganic and (bio-)organic chemistry provides a rich wellspring of novel reactivity that expands substantively the scope of transformations that can be realized.[115]

The search for inherently benign synthetic methodologies represents a key strategy for the development of clean, sustainable, and cost-effective processes. Apart from optimizing known processes, entirely new synthetic procedures have to be developed, e.g. direct functionalization of carbon—hydrogen bonds, selective oxidation with molecular oxygen, and the use of carbon dioxide as highly desirable transformations that are not yet included in the toolbox of practical syntheses.

Catalysis will continue to play an integral role in shaping the future of organic synthesis, i.e. catalysis is the key to sustainability. The way forward is full integration of catalytic methodologies in organic synthesis. There is also a pressing need for integration of the different modes of catalysis: homogeneous, heterogeneous, and enzymatic. This would lead to a synergistic cross-fertilization of ideas. The ultimate synergy is the integration of chemo- and biocatalytic steps to afford catalytic cascades that are reminiscent of what occurs in the living cell.[116]

Cell Factory Design. Apart from the chemical technology developments mentioned above, there will be an increasing impact from reengineering metabolic pathways on the way multistep organic syntheses will be carried out in the fine chemical industry. For the next generation of microbial conversions, the challenge of molecular biology is to:

(1) improve productivity in terms of carbon-source efficiency of microorganisms, towards a much higher product/biomass ratio;

(2) broaden the scope of products, from natural products toward the modified products often required for drugs, for example;

(3) reengineer metabolic pathways as the most efficient way to reach these two goals.

These steps should free fermentation processes from excessively using renewables that end up in undesired, low-value biomass byproducts and should minimize additional chemical modification steps to obtain the final product.

In particular, the appropriate integration of microbial, enzymatic, and chemical catalytic transformations in a cascade mode may be seen as the ultimate tool for sustainable fine chemicals processing. In this respect, the increasing insight into the precise functioning of living cells as high-tech miniature factories will surely be a source of inspiration for the development of novel sophisticated processing devices for the fine chemical industry.

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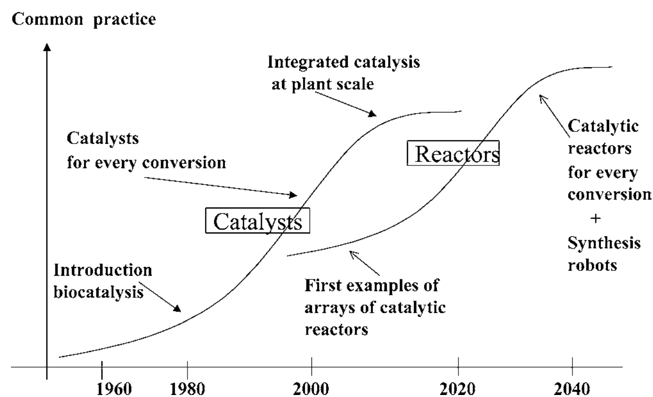

Figure 12. **S-curves for developments in catalysis in fine chemicals syntheses.**

Conclusions

The concepts and various examples given show that we may foresee a renaissance in synthesis methods by both the integration of bio- and organic syntheses and the development of multichemocatalytic conversion methods. Fine chemicals of the future will be produced by cascade multistep catalytic procedures without intermediate recovery steps, including:

(1) multistep (catalytic) conversions by appropriate combinations of acids, bases, metals, enzymes, and microorganisms;

(2) novel, mutually compatible, bio- and chemocatalytic synthetic methods and procedures that allow operation under standard reaction conditions and solvents;

(3) convergence of various, more broadly applicable, cascades into a final cascade transformation to the desired end product to gain flexibility as well as to avoid selectivity problems of poly-step linear cascades;

(4) integration of combined (catalytic) conversions with in situ product separation techniques in continuous processing;

(5) use of selective isotope labeling and NMR as the analytical tools to study whole-reaction mixtures of such complex transformations in situ;

(6) full deployment of high throughput screening techniques for catalyst discovery and development as well as large-scale application of parallel experimentations in development and optimization of synthesis routes.

Whereas we might in principle have a catalyst available today for every synthetic conversion (see Figure 12), it will probably take another 10−20 years before enjoying the full potential of cascades of catalysis at industrial scale, since we have to realize that:

(1) either only transformations with very high selectivity are applicable or that in situ byproduct separation should be accomplished by reactor design;

(2) even trace amounts of materials might inhibit or poison subsequent catalytic conversion steps, asking for compartmentalization and medium engineering;

(3) much better mutually compatible reaction conditions for a wide range of transformations are required, asking for new (catalytic) synthetic methods and cell factory design.

In reactor design for the fine chemical industry a still longer course of development has to be completed. The gap between today's practice (i.e. mainly batch-type reactors) and the alternatives shown in this review is still huge and wide. In fact it might be to early to translate the present developments in reaction/reactor engineering to the fine chemical reactor of the future. Integration of reactor and catalyst, however, is a clear trend as is full-scale application of microsystems technologies. It is tempting to foresee a future in which a catalytic device is available for every conceivable molecular conversion. Combined with more advanced retrosynthetic schemes this would allow highly automated or even robotized multistep syntheses of new molecules in the earliest stages of product development. At the same time these devices could be used to turn around the large-scale manufacturing from volume-guided to adding numbers of (micro-) reactors. Scale-up through pilot plants would no longer be required, allowing shorter process development times. Alternatively, the combination of microsystems technologies and high throughput parallel experimentations in catalytic microreactor devices at laboratory scale could well be used to prepare batch-scale production in a much more reliable way. Recent advances in down scaling rules[117] from batch reactors to laboratory protocols could very well stimulate this scenario in which pilot plants could also be avoided as well as investments in new manufacturing equipment.

Last, but not least, cascade and multistep one-pot conversions give various paths for safer processing designs, for instance by reduction of the handling and storage of process intermediates, by replacement of hazardous chemicals, and by more in situ creation and use for highly toxic chemicals, i.e. by shifting to just-in-time production.[118] As the intermediate products of multistep organic syntheses are often highly active or toxic compounds, cascade processes will prevent the exposure of operators and the environment to such chemicals.

Combined (catalytic) reactions without intermediate recovery steps comprise one of the important future directions for carrying out sustainable organic syntheses on a natural way with inherently safer designs.

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