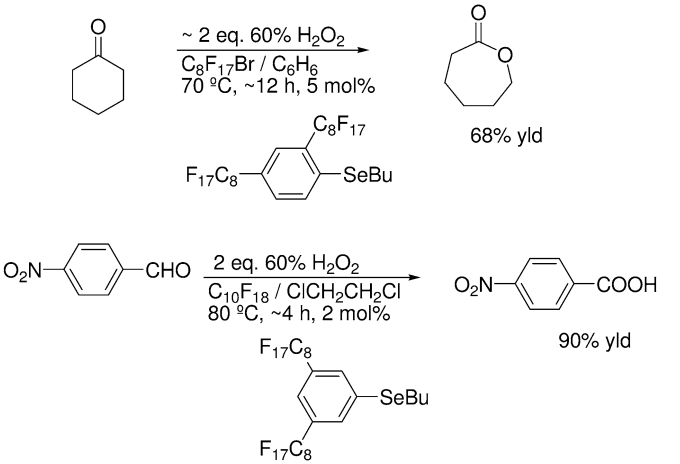


Fig. 4.84 Fluorous phase BV-oxidation using fluorous selenium catalysts.

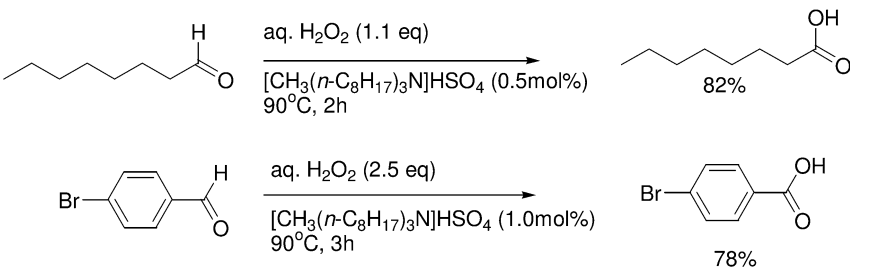


Fig. 4.85 Quaternary ammonium salts as catalyst for BV-oxidation with H_2O_2.

Heterogeneous catalysts are also active for the Baeyer-Villiger oxidation with H_2O_2. One of the best – based on 1.6 wt% tin in zeolite beta – was developed in the group of Corma [250, 251]. It provides a green method for BV oxidations in that it utilizes hydrogen peroxide as the oxidant, a recyclable catalyst, and avoids the use of chlorinated hydrocarbon solvents. It is believed that the Sn Lewis acid sites solely activate the ketone for BV-reaction, and leave hydrogen peroxide non-activated. Corma proposed that, in this way, side-reactions, such as epoxidation, which require electrophilic activation of hydrogen peroxide are largely avoided. Oxidation of bicyclo[3.2.0]hept-2-en-6-one with hydrogen peroxide in the presence of tin zeolite beta gave selective oxidation of the ketone, leaving the olefin intact. The tin catalyst may be particularly effective, because it can expand its coordination sphere to 6, thereby providing room for coordination of both the ketone and the hydroxide leaving group.

Surprisingly, both in the oxidation of bicyclo[3.2.0]hept-2-en-6-one and of dihydrocarvone (not shown) isomeric lactones formed by migration of the secondary carbon, as is usual in Baeyer-Villiger reactions, were not observed. Other

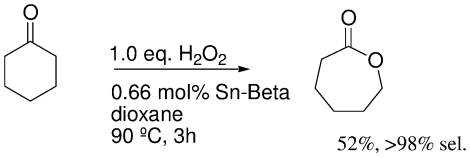


Fig. 4.86 Sn-Beta catalyzed BV-oxidation.

ketones (e.g. cyclohexanone, Fig. 4.86) were oxidized with remarkable selectivity (>98%) considering the reaction conditions and the catalyst was recycled several times without loss of activity. Dioxane or the more attractive, methyl-*tert*-butyl ether, were used as solvents in these transformations.

Also very important is the asymmetric version of the BV-reaction. In this area biocatalysts are very promising, see Section 4.7.

4.5.5
Oxidation of Phenols

The aerobic oxidation of phenols in the presence of cobalt-Schiff's base complexes as catalysts is facilitated by (electron-donating) alkyl substituents in the ring and affords the corresponding *p*-quinones, e.g. the Vitamin E intermediate drawn in Fig. 4.87. When the *para*-position is occupied the reaction may be directed to the *ortho*-position [252, 253]. Copper compounds also mediate this type of oxidation, e.g. the Mitsubishi Gas process for the Vitamin E intermediate [254].

Phenol undergoes hydroxylation with H_2O_2 in the presence of Brønsted ($HClO_4$, H_3PO_4) or Lewis acid (TS-1) catalysts, affording a mixture of catechol and hydroquinone [255]. The reaction proceeds via a heterolytic mechanism involving an electrophilic Ti–OOH species or, in the case of Brønsted acids, perhaps even a hydroxy cation. On the other hand, catalysis by Fe^{II}/Co^{II} (Brichima process) involves a homolytic mechanism and hydroxyl radical intermediates [255] (see Table 4.10).

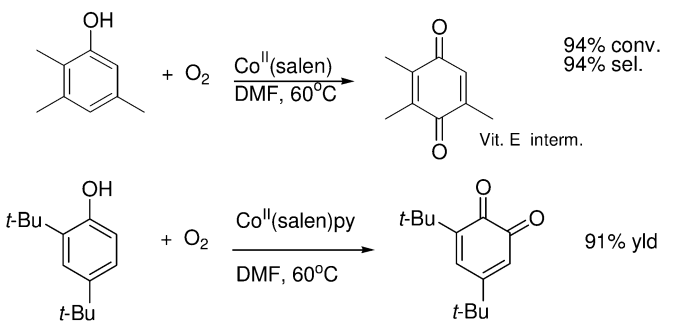


Fig. 4.87 Aerobic oxidation of phenols.

Table 4.10 Comparison of phenol conversion processes [255].

Process (catalyst)	Rhone-Poulenc ($HClO_4$, H_3PO_4)	Brichima (Fe^{II}/Co^{II})	Enichem (TS-1)
Phenol conversion %	5	10	25
Selectivity on phenol %	90	80	90
Selectivity on H_2O_2	70	50	70
Catechol/hydroquinone	1.4	2.3	1.0

4.5.6
Oxidation of Ethers

Ethers are readily autoxidized at the α-position to the corresponding α-hydroperoxyethers, which is of no synthetic utility, but important to realize when ethers are evaporated to leave an explosive residue of α-hydroperoxyethers behind. Ethers are oxidized with stoichiometric amounts of high valent metal oxides such as RuO_4 [256], CrO_3 [257] and $KMnO_4$ [258], but very few catalytic oxidations are known and these generally pertain to activated, e.g. benzylic, substrates. One example is the NHPI [259] catalyzed oxidation of methyl benzyl ether to the corresponding acetate (Fig. 4.88).

Recently a convenient and selective Ru/hypochlorite oxidation protocol for the selective transformation of a series of ethers and alcohols was reported [260]. The catalytic systems described in the literature, especially regarding ether oxidation, have limited reproducibility and/or require an excess of terminal oxidant, i.e. NaOCl solutions (household bleach). When the pH is maintained at 9–9.5 the reactions proceed smoothly, using the theoretical amount of hypochlorite, with fast, complete conversion of substrate and high selectivity to the target molecules at low concentration of Ru precursor. A variety of Ru precursors can be used, although it was found that $[Pr_4N][RuO_4]$ (TPAP) gave the best results. The catalyst can also be recycled with negligible loss of selectivity and minimum loss of activity, which can be further optimised by scale-up. In the oxidation of dibutyl ether, only 0.25 mol% of TPAP was used in ethyl acetate as the solvent, leading to 95% yield and 97% selectivity towards butyl butyrate (Fig. 4.89).

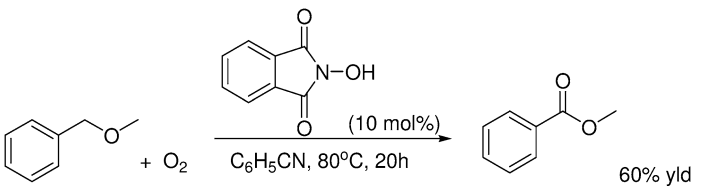


Fig. 4.88 NHPI catalyzed oxidation of ethers.

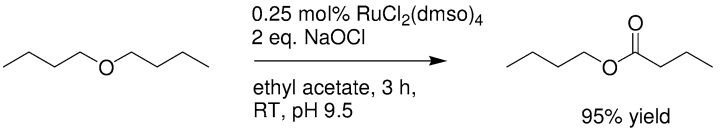


Fig. 4.89 Green protocol for TPAP/NaOCl catalyzed oxidation of ethers.

4.6 Heteroatom Oxidation

Autoxidations of substrates containing heteroatoms tend to be complex and non-selective processes. Nonetheless, a number of selective catalytic reactions with hydrogen peroxide are known.

4.6.1 Oxidation of Amines

A large number of different nitrogen-containing compounds such as primary, secondary and tertiary amines, *N*,*N*-dimethylhydrazones, hydroxylamines, pyridines, etc. can be oxidized with e.g. hydrogen peroxide, leading to an equally vast number of different products. The presence of an α-hydrogen may have a major influence on the outcome of the reaction. Furthermore, a distinction can be made between oxidations involving high-valent peroxometal, or oxometal species and oxidative dehydrogenations mediated by low-valent transition metal catalysts. Nonetheless, with the right choice of solvent and equivalents of oxidants the reactions can be directed to a certain extent.

4.6.1.1 Primary Amines

Primary amines are dehydrogenated by high-valent oxometal species to give nitriles, or imines depending on the number of available α-hydrogens. Oxidation of the amine via peroxometal-intermediates (e.g. with MTO, Na_2MoO_4,

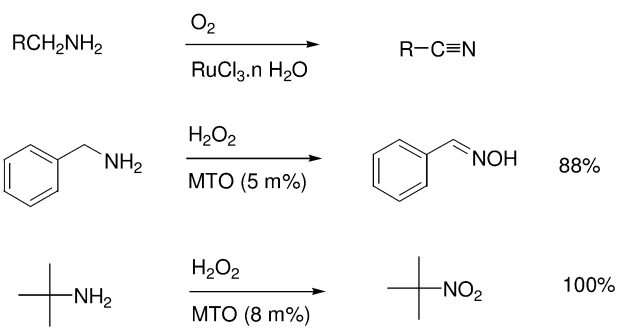


Fig. 4.90 Oxidation of primary amines.

Na_2WO_4, $NaVO_3$ in combination with H_2O_2) leads to the formation of oximes [261, 262], or in the absence of α-hydrogens even to nitro-compounds (Fig. 4.90) [263].

4.6.1.2 **Secondary Amines**

Peroxometal-forming catalysts e.g. MTO, SeO_2 or Na_2WO_4, catalyze the oxidation of secondary amines to nitrones via the corresponding hydroxylamines, Fig. 4.91 a [262]. In the absence of α-hydrogens these substrates will also give nitroxides ($R_2NO^{\bullet}$), see Fig. 4.91 b [263]. Here again oxometal complexes react differently, giving the dehydrogenated imine rather than the nitrone [122, 264], as is illustrated in Fig. 4.92.

4.6.1.3 **Tertiary Amines**

Similarly, for tertiary amines a distinction can be made between oxometal and peroxometal pathways. Cytochrome P450 monooxygenases catalyze the oxidative *N*-demethylation of amines in which the active oxidant is a high-valent oxoiron species. This reaction can be mimicked with some oxometal complexes (Ru^{V}=O), while oxidation via peroxometal complexes results in oxidation of the N atom (Fig. 4.93 a and b) [261]. A combination of MTO/hydrogen peroxide can

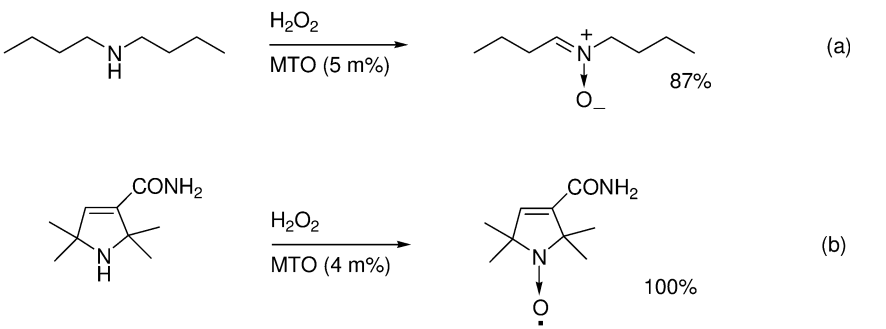


Fig. 4.91 MTO catalyzed oxidation of secondary amines.

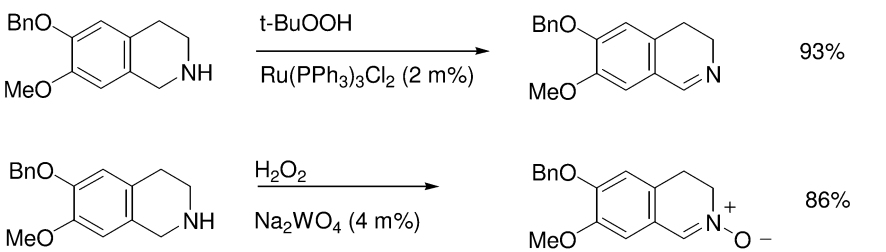


Fig. 4.92 Oxidation of secondary amines via Ru and W catalysts.

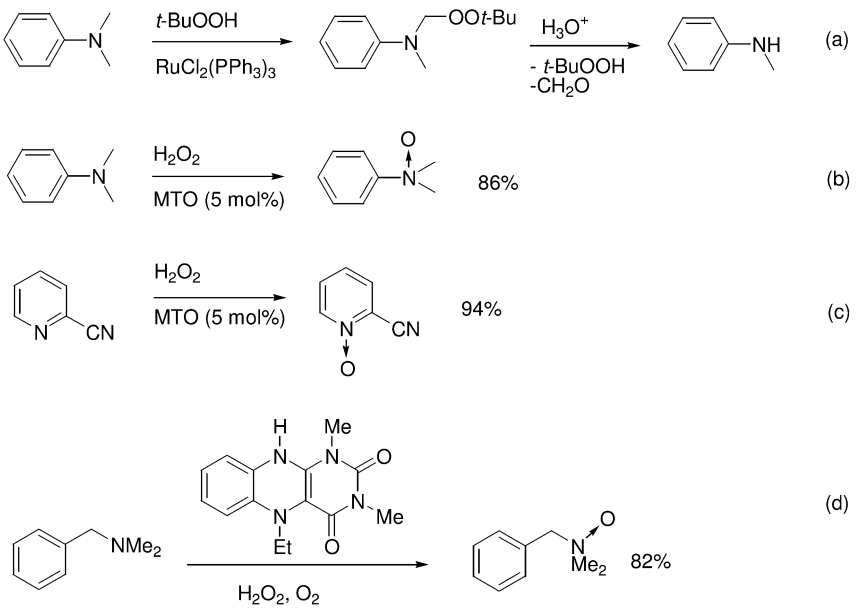


Fig. 4.93 Oxidation of tertiary amines.

also oxidize pyridines to pyridine *N*-oxides as was recently published by Sharpless et al. [265]. A rare example of clean oxidation of tertiary amines without metal compounds was reported by Bäckvall et al. [266] using flavin as the catalyst (Fig. 4.93 d).

4.6.1.4 **Amides**

Ruthenium oxo compounds catalyze the oxidation of amides, e.g. *β*-lactams, at the position *α* to the nitrogen, using peracetic acid as the oxidant, presumably via oxoruthenium intermediates [264].

4.6.2 **Sulfoxidation**

Dialkyl sulfides are readily oxidized by e.g. H_2O_2 in the presence of metal catalysts to give the corresponding sulfoxides. In turn the sulfoxides can be further oxidized to the corresponding sulfones R–S(O)$_2$–R′ with excess peroxide. Due to the polarity of the S–O bond the second oxidation step is more difficult for electrophilic oxidation catalysts. This is especially important in the oxidative destruction of mustard gas where the corresponding sulfoxide is relatively harmless, but the sulfone quite toxic again [267]. In order to see which catalyst is likely to give "overoxidation" of the sulfoxide the thianthrene 5-oxide (see Fig. 4.94) is an excellent probe to test catalysts active in oxygen transfer reactions for whether

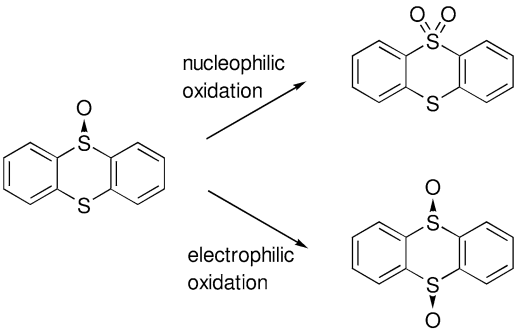


Fig. 4.94 Nucleophilic vs. electrophilic S oxidation pathways.

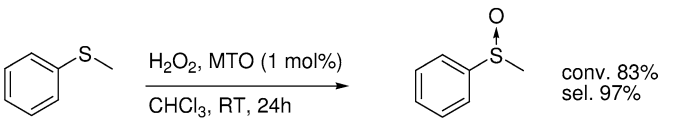


Fig. 4.95 MTO/H_2O_2 catalyzed sulfide oxidation.

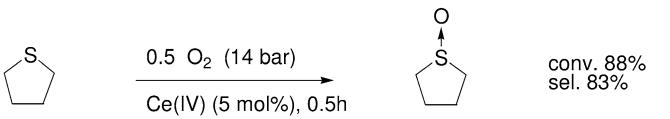


Fig. 4.96 Sulfide oxidation using air as the oxidant.

they are nucleophilic or electrophilic in character. Catalytic oxidations with hydrogen peroxide are generally performed with early transition elements, e.g. Mo(VI) [268], W(VI), Ti(IV), V(V), Re(V) [269] and Re(VII) (=MTO, Fig. 4.95) [270] or seleninic acids as catalysts [271] and involve peroxometal pathways. Sulfides can also be oxidized with dioxygen, using a cerium (IV)/cerium (III) redox couple [271], via a homolytic mechanism involving a sulfonium radical cation intermediate (Fig. 4.96).

4.7 Asymmetric Oxidation

Owing to the instability of many (chiral) ligands under oxidative reaction conditions, asymmetric oxidation is not an easy reaction to perform. However, much progress has been achieved over the last decades. Because of the relatively low volumes and high added value of the products asymmetric oxidation allows the use of more expensive and environmentally less attractive oxidants such as hypochlorite and *N*-methylmorpholine-*N*-oxide (NMO).

4.7.1
Asymmetric Epoxidation of Olefins

Chiral epoxides are important intermediates in organic synthesis. A benchmark classic in the area of asymmetric catalytic oxidation is the *Sharpless* epoxidation of allylic alcohols in which a complex of titanium and tartrate salt is the active catalyst [273]. Its success is due to its ease of execution and the ready availability of reagents. A wide variety of primary allylic alcohols are epoxidized in >90% optical yield and 70–90% chemical yield using *tert*-butyl hydroperoxide as the oxygen donor and titanium-isopropoxide-diethyltartrate (DET) as the catalyst (Fig. 4.97). In order for this reaction to be catalytic, the exclusion of water is absolutely essential. This is achieved by adding 3 Å or 4 Å molecular sieves. The catalytic cycle is identical to that for titanium epoxidations discussed above (see Fig. 4.20) and the actual catalytic species is believed to be a 2:2 titanium(IV) tartrate dimer (see Fig. 4.98). The key step is the preferential transfer of oxygen from a coordinated alkylperoxo moiety to one enantioface of a coordinated allylic alcohol. For further information the reader is referred to the many reviews that have been written on this reaction [274, 275].

The applicability of the "Sharpless" asymmetric epoxidation is however limited to functionalized alcohols, i.e. allylic alcohols (see Table 4.11). The best method for non-functionalized olefins is the Jacobsen-Kaksuki method. Only a few years after the key publication of Kochi and coworkers on salen-manganese complexes as catalysts for epoxidations, Jacobsen and Kaksuki independently described, in 1990, the use of chiral salen manganese(III) catalysts for the synthesis of optically active epoxides [276, 277] (Fig. 4.99). Epoxidations can be carried out using commercial bleach (NaOCl) or iodosylbenzene as terminal oxidants and as little as 0.5 mol% of catalyst. The active oxidant is an oxomanganese(V) species.

Over the years further tuning of the ligand by varying the substituents in the aromatic ring has led to >90% *ee* for a number of olefins [278]. Improvements were also achieved by adding amine-*N*-oxides such as 4-phenylpyridine-*N*-oxide

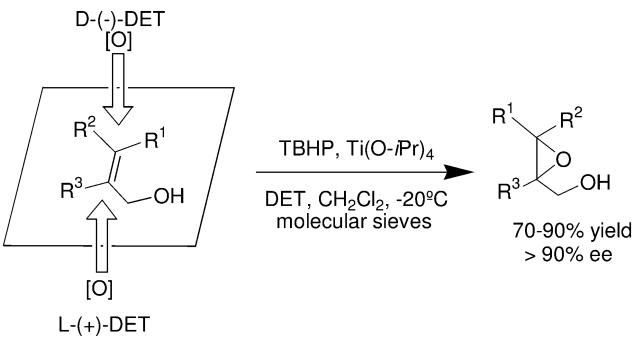


Fig. 4.97 The "Sharpless" catalytic asymmetric epoxidation

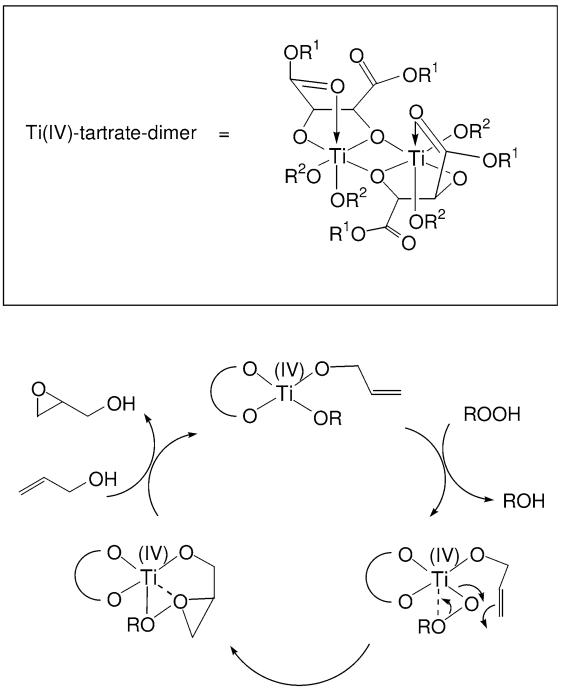
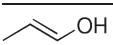
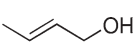
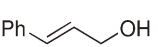
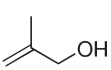
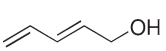


Fig. 4.98 The catalytic cycle of the "Sharpless" catalytic asymmetric epoxidation.

Table 4.11 Sharpless asymmetric epoxidation of allylic alcohols.[a)]

Substrate	Yield (%)	*ee* (%)
	65	90
	70	96
	89	>98
	50	>95
	40	95

a) Results with 5 mol% of catalyst, see Ref. [273].

which serve as axial ligands in the active oxomanganese(V) species. The method generally gives good results for cis-disubstituted olefins, whereas trans-disubstituted olefins are less suitable substrates (see Table 4.12). On the other hand, chromium-salen complexes catalyze the asymmetric epoxidation of trans-substituted olefins in reasonable *ee*s (see Fig. 4.100) using iodosylarenes as oxidants [279].

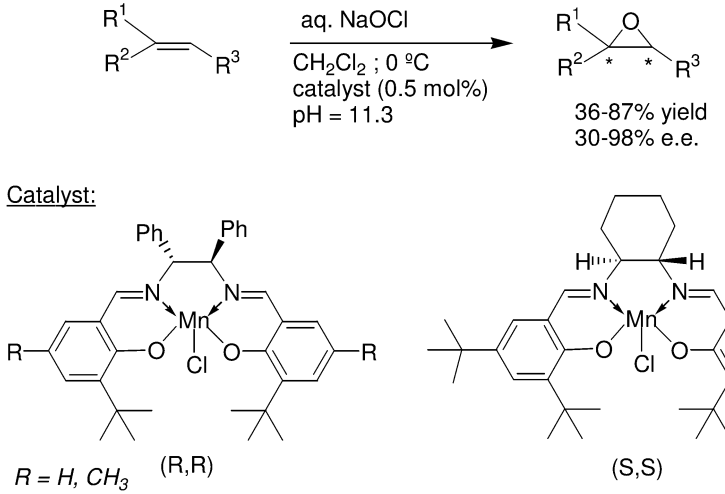
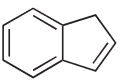
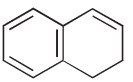
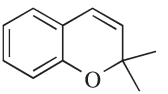
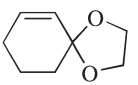
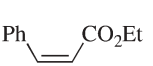


Fig. 4.99 The Jacobsen-Katsuki asymmetric epoxidation of unfunctionalized olefins.

Table 4.12 Jacobsen-Katsuki asymmetric epoxidation of unfunctionalized olefins.

Olefin	Method [a]	Equiv. catalyst	Isolated yield (%)	*ee* (%)
	A	0.04	84	92
	A	0.01	80	88
	B	0.04	67	88
	A	0.02	87	98
	B	0.15	63	94
	B	0.08	67	97

a) Method A: NaOCl, pH 11.3, CH_2Cl_2, 0 °C. Method B: same as A + 0.2 eq. 4-phenylpyridine-*N*-oxide.

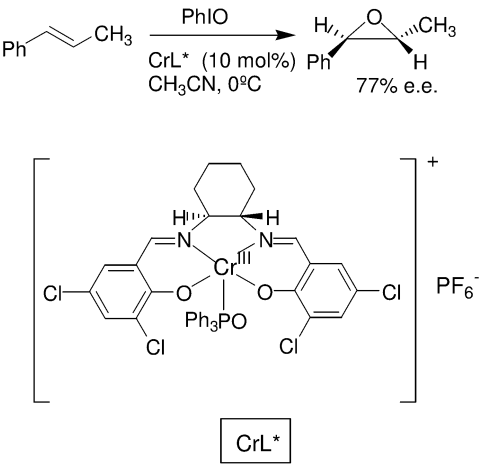


Fig. 4.100 Cr-salen catalyzed asymmetric epoxidation.

The recent reports of Katsuki and Beller using Ti- [280, 281] and Ru-based [282] complexes for asymmetric epoxidation with H_2O_2, have however changed the state-of-the-art in the area of metal-catalyzed asymmetric epoxidations. Especially for titanium in combination with reduced salen-type ligands, excellent stereochemical control was achieved for terminal olefins using hydrogen peroxide as the oxidant (see Fig. 4.101). Also with ruthenium, notorious for its catalase activity, excellent yields and enantioselectivities were obtained for cis- and activated olefins. In Table 4.13 an overview is given of the performance of titanium and ruthenium complexes as catalysts with hydrogen peroxide.

For titanium, only 1 mol% of catalyst Ti(salalen) (Table 4.13) and 1.05 eq. H_2O_2 are required to obtain high yields and selectivities. 1,2-Dihydronaphthalene is an activated cis-olefin, which obviously gives the best results (yield and enantioselectivity of over 98%) using little or no excess of hydrogen peroxide. However also for simple styrene 93% *ee* can be achieved using this chiral titanium complex. What is most striking is the result obtained for 1-octene. For this simple non-activated olefin a reasonable 82% *ee* could be attained. Titanium-salan (see Table 4.13) as a catalyst requires higher loadings (5 mol%), but its ease of synthesis and *in situ* synthesis from Ti(O*i*Pr)$_4$ and salan ligand, makes this a very promising catalyst for practical applications.

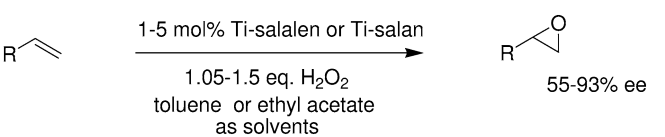
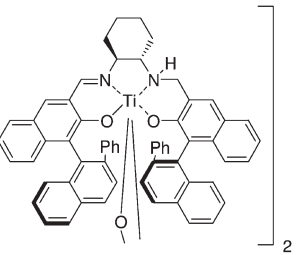
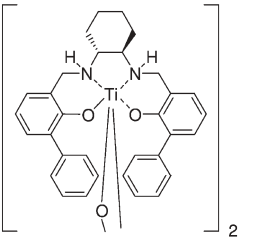


Fig. 4.101 Ti-catalyzed asymmetric epoxidation of olefins using aqueous hydrogen peroxide as the oxidant.

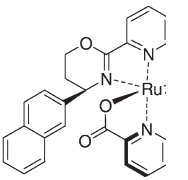
ble 4.13 Comparison of metal catalysts for the asymmetric epoxidation of alkenes using aqueous H_2O_2.



di-μ-oxo titanium(salalen) -**1**



di-μ-oxo titanium(salan) -**2**



Ru(pyboxazine)(pydic) -**3**

bstrate	Catalyst	Catalyst loading (mol%)	Eq. 30% aqueous H_2O_2	Yield epoxide (%)	*ee* epoxide (%)	Ref.
	Ti(salalen)-**1** [a)]	1	1.05	90	93	280
	Ti(salan)-**2** [b)]	5	1.5	47	82	281
	Ru(pyboxazine)(pydic)-**3** [c), d)]	5	3	85	59	282
	Ti(salalen)-**1** [a)]	1	1.05	70	82	280
	Ti(salan)-**2** [b)]	5	1.5	25	55	281
	Ti(salalen)-**1** [a)]	1	1.05	99	99	280
	Ti(salan)-**2** [b)]	5	1.5	87	96	281
	Ru(pyboxazine)(pydic)-**3** [c)]	5	3	95	72	282

RT, 12–48 h, CH_2Cl_2 as solvent.
25 °C, 6–24 h, CH_2Cl_2 as solvent.
RT, 12 h, 2-methylbutan-2-ol as solvent.
20 mol% acetic acid was added.

Mukaiyama has reported the use of manganese complexes for chiral epoxidations using a combination of molecular oxygen and an aldehyde as the oxidant [283]. With salen-manganese(III) complexes and pivaldehyde/oxygen the corresponding epoxides of several 1,2-dihydronaphthalenes were obtained in very good yields (77 to 92% *ee*) in the presence of *N*-alkyl imidazole as axial ligand. Altering the ligand structure from salen derivatives to optically active *β*-ketoimine-type ligands, gave the novel manganese catalysts in Fig. 4.102 which oxidize phenyl-conjugated olefins, such as dihydronaphthalenes and cis-*β*-methyl styrene in 53 to 84% *ee* [284]. The enantiofacial selection in these manganese-catalyzed epoxidations is opposite to that obtained with sodium hypochlorite or iodosylbenzene as the primary oxidant in the Jacobsen-Katsuki epoxidation. Apparently the catalytically active species differs from the putative oxomanganese(V) complex in the latter processes. An acylperoxomanganese complex was proposed as the active oxidant, i.e. a peroxometal rather than an oxometal mechanism.

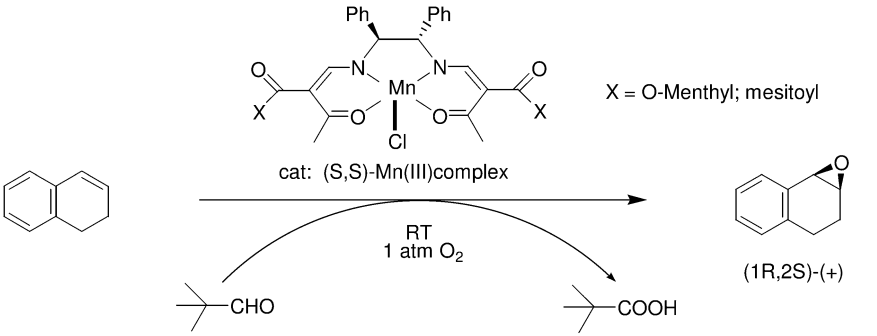


Fig. 4.102 Enantioselective epoxidation of phenyl-conjugated olefins employing aldehyde and molecular oxygen as the oxidant.

A very different, non-metal based, approach, for the synthesis of trans-disubstituted chiral epoxides is the use of the highly reactive chiral dioxiranes (Fig. 4.103). These oxidants can be generated *in situ* by using potassium persulfate as the primary oxidant. Although this method requires the use of 20–30 mol% of dioxirane precursor, the reaction proceeds with high chemical yields and *ee*s over 90% for a wide variety of trans-disubstituted olefines [285]. The mechanism is given in Fig. 4.104, and involves a spiro transition state. More recently, it was shown that the persulfate can be replaced by H_2O_2 in CH_3CN, which generates the peroxyimidic acid (Payne reagent) *in situ* [286].

Another method is to use poly-L-amino acids as catalysts in alkaline media (Julia-Colanna epoxidation) for the asymmetric epoxidation of chalcones and other electron-poor olefins with H_2O_2 [287]. SmithKline Beecham workers used this method (see Fig. 4.105) as a key step in the synthesis of a leukotriene antagonist, although it required 20 equivalents of H_2O_2 and 12 equivalents of NaOH, based on substrate [288]. The mechanism probably involves the asym-

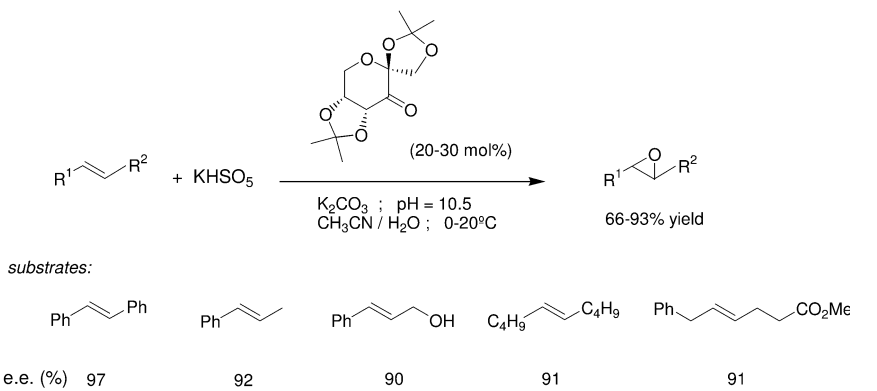


Fig. 4.103 Efficient asymmetric epoxidation of trans-alkenes.

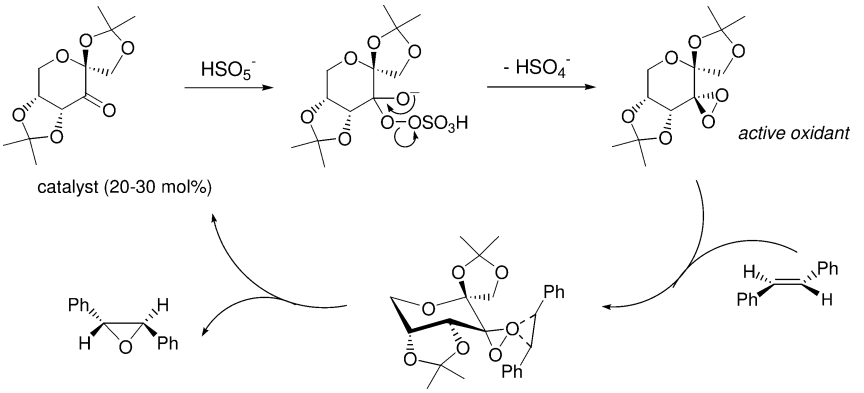


Fig. 4.104 Catalytic cycle of asymmetric epoxidation via chiral dioxiranes.

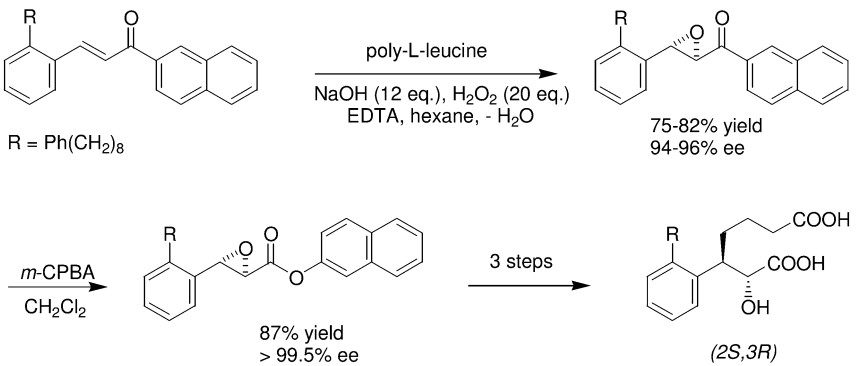


Fig. 4.105 Asymmetric epoxidation of chalcones.

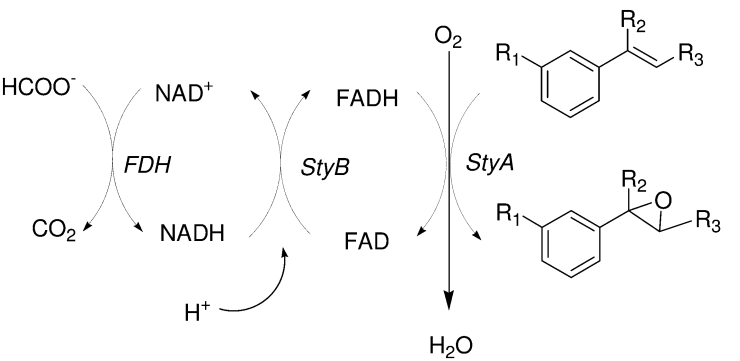
metric addition of a hydroperoxy anion (HOO^-) to the olefinic double bond, followed by epoxide ring closure. The poly-L-amino acid acts as a chiral phase transfer catalyst and may be considered as a synthetic enzyme mimic. Further improvements of the Julia-Colonna epoxidation have been achieved by using sodium percarbonate as the oxidant and recyclable silica-supported polyamino acids as catalysts in dimethoxyethane as solvent [289].

Finally, enzymes themselves can be used for the direct epoxidation of olefins. As already outlined in Section 4.2, peroxidases (which can directly apply hydrogen peroxide as the oxidant) as well as monooygenases are available. Because of its ease of application, chloroperoxidase (CPO) from *Caldariomyces fumago* is very promising. It is commercially available and displays a reasonable substrate range [290]. The main problem is its instability under reaction conditions and the sluggish reaction rates. Typical turnover rates are 0.1–2 s^{-1}. The epoxidation of styrene in the presence of 0.8% of surfactant is, at 5.5 s^{-1} the fastest on record [291]. The advantage is that the *ee*s in most cases are always good to excel-

lent. For example 2-heptene could be oxidized with 1700 turnovers relative to enzyme in 30% t-BuOH. The *ee* of the resulting epoxide was 96%. The total turnover number could be further increased in this reaction (from 1700 to 11500) when the hydrogen peroxide was generated *in situ* by the glucose oxidase-mediated reaction of glucose and oxygen. A wide range of 2-methyl-1-alkenes could also be oxidized with *ee*s ranging from 95 to 50% [292]. 1-Alkenes with the exception of styrene are suicide reactants that alkylate the heme in native CPO. The latter problem can be circumvented by using certain mutants [293]. Styrene resulted in only 49% *ee*, for this substrate the styrene monooxygenases seem to be better suited.

Recently, the first asymmetric cell-free application of styrene monooxygenase (StyAB) from *Pseudomonas* sp. VLB120 was reported [294]. StyAB catalyses the enantiospecific epoxidation of styrene-type substrates and requires the presence of flavin and NADH as cofactor. This two-component system enzyme consists of the actual oxygenase subunit (StyA) and a reductase (StyB). In this case, the reaction could be made catalytic with respect to NADH when formate together with oxygen were used as the actual oxidant and sacrificial reductant respectively. The whole sequence is shown in Fig. 4.106. The total turnover number on StyA enzyme was around 2000, whereas the turnover number relative to NADH ranged from 66 to 87. Results for individual substrates are also given in Fig. 4.106. Excellent enantioselectivities are obtained for α- and β-styrene derivatives.

Recently it was shown that the flavin (FAD in Fig. 4.106) could be directly regenerated by the organometallic complex $Cp^*Rh(bpy)(H_2O)]^{2+}$ and formate. In



m-chlorostyrene (R_1=Cl, R_2,R_3=H)	73% yld.	90.5% conv.	>99.9% ee
α-methyl-styrene (R_2=CH_3, R_1,R_3=H)	75% yld.	87.9% conv.	98.1% ee
β-methyl-styrene (R_1,R_2=H, R_3=CH_3)	87% yld.	95.3% conv.	99.7% ee

Fig. 4.106 Biocatalytic epoxidation with styrene monooxygenase including cofactor regeneration.

this way the reaction becomes simpler, because only enzyme (StyA), FAD and Rh-complex are required. The resulting turnover number on Rh ranged from 9 to 18 depending on the substrate [295].

4.7.2
Asymmetric Dihydroxylation of Olefins

Following their success in asymmetric epoxidation, Sharpless and coworkers developed an efficient method for the catalytic asymmetric dihydroxylation of olefins. The method employs catalytic amounts of OsO_4 and derivatives of cinchona alkaloids as chiral ligands together with *N*-methylmorpholine-*N*-oxide (NMO) as the primary oxidant, see Fig. 4.107 [296]. The use of osmium(VIII) complexes as catalysts leads to stereospecific 1,2-cis addition of two OH groups to the olefin. The reaction greatly benefits from ligand accelerated catalysis, i.e. the ligation of osmium by the alkaloid enhances the rate of reaction with the alkene by one to two orders of magnitude relative to the reaction without ligand. The reaction is complicated by the fact that two catalytic cycles are possible, see Fig. 4.108 [297]. The primary cycle proceeds with high face selectivity and involves the chiral ligand in its selectivity determining step, the formation of the osmium(VI)glycolate. The latter is oxidized by the primary oxidant to the osmium(VIII)glycolate which is hydrolyzed to the product diol with concomitant

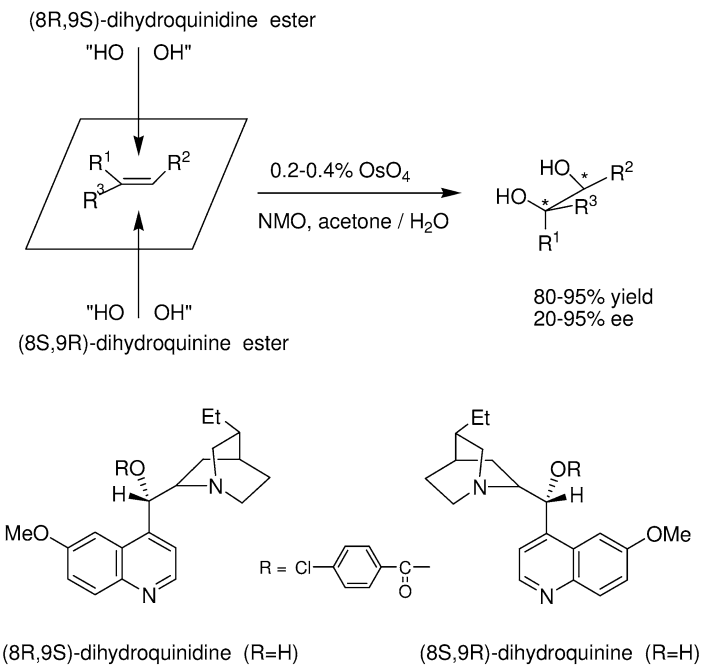


Fig. 4.107 Asymmetric dihydroxylation of alkenes.

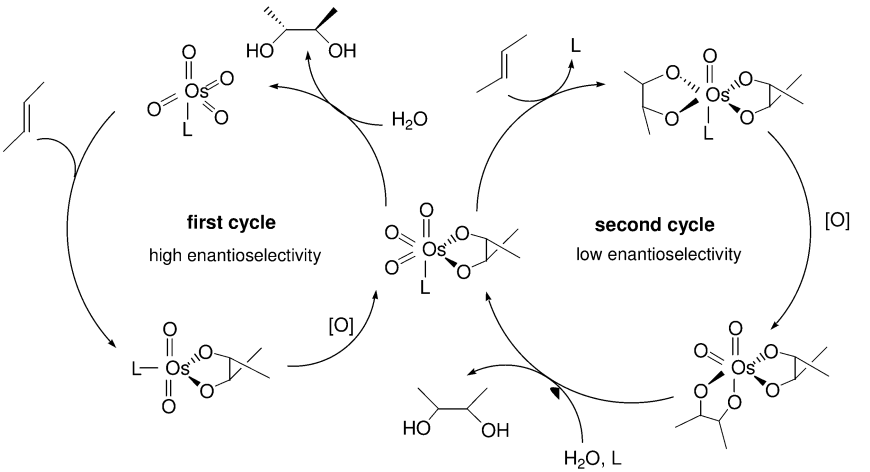


Fig. 4.108 Mechanism of asymmetric dihydroxylation.

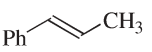
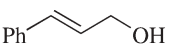
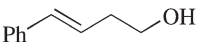
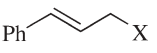
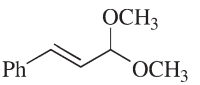
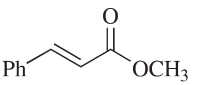
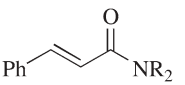
generation of the catalyst. In the second catalytic cycle the osmium(VIII)glycolate reacts with a second molecule of olefin, displacing the chiral ligand and resulting in poor overall enantioselectivity. The desired pathway involves hydrolysis of the osmium(VI)glycolate in competition with coordination of another olefin molecule. Therefore, slow addition of olefin is essential to obtain high *ee*s.

Alternatively, the process can be performed with $K_3Fe(CN)_6$ as the stoichiometric oxidant in *tert*-butanol/water mixtures [298]. In this case the olefin osmylation and osmium reoxidation steps are uncoupled, since they occur in different phases, resulting in improved enantioselectivities. However, from a practical point of view, the improved enantioselectivities (about 5–10% *ee*) are probably offset by the use of a less attractive oxidant.

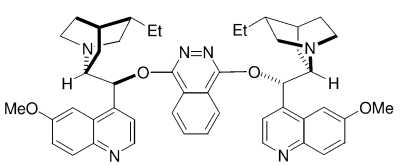
When using NMO as the oxidant, on the other hand, the reduced form is readily recycled by oxidation with H_2O_2. The asymmetric dihydroxylation is successful with a broad range of substrates, in contrast to the Sharpless asymmetric epoxidation which is only suitable for allylic alcohols (Table 4.14). Further development of the system has led to the formulation of a reagent mixture, called AD-mix based on phthalazine-type ligands, which contains all the ingredients for the asymmetric dihydroxylation under heterogeneous conditions including OsO_4 [299], see Fig. 4.109. One equivalent of methanesulfonamide is added to accelerate the hydrolysis of the osmium(VI)glycolate and thus to achieve satisfactory turnover rates.

Beller et al. [85] recently described the aerobic dihydroxylation of olefins catalyzed by osmium at basic pH, as mentioned above. When using the hydroquinidine and hydroquinine bases, they were able to obtain reasonable enantioselectivities (54% *ee* to 96% *ee*) for a range of substrates. An alternative route towards enantiopure diols, is the kinetic resolution of racemic epoxides via enantioselective hydrolysis catalyzed by a Co(III)salen acetate complex, developed by Jacob-

Table 4.14 Comparison of asymmetric epoxidation (AE) and asymmetric dihydroxylation (AD) according to refs. [273] and [299].

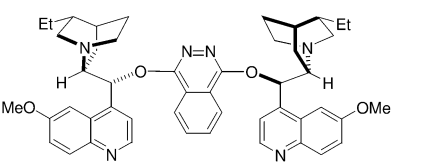
Alkene	Substrate for	
	AD	**AE**
	>95% *ee*	NR
	80% *ee*	>95% *ee*
	>95% *ee*	30–50% *ee*
 *X=OAc, OCH_2Ph, N_3, Cl*	>95% *ee*	NR
	>95% *ee*	NR
	>95% *ee*	NR
	>95% *ee*	NR

NR = non-relevant.



$(DHQD)_2$-PHAL,

ligand used in AD-mix-β



$(DHQ)_2$-PHAL,

ligand used in AD-mix-α

Fig. 4.109 Ligands used in commercial catalysts for asymmetric dihydroxylation.

sen [300]. In this case the maximum theoretical yield of the enantiomerically pure diol is 50%, compared with 100% in the asymmetric dihydroxylation method. Nonetheless, its simplicity makes it a synthetically useful methodology.

4.7.3
Asymmetric Sulfoxidation

In the area of metal catalyzed asymmetric sulfoxidation there is still much room for improvement. The most successful examples involve titanium tartrates, but at the same time often require near stoichiometric quantities of catalysts [301, 302]. Recently, this methodology has been successfully used for the production of (*S*)-Omeprazole by AstraZeneca [303] (see Fig. 4.110). A modified Kagan-procedure [302] was applied, using cumene hydroperoxide as the oxidant. Another example is the sulfoxidation of an aryl ethyl sulfide, which was in development by Astra Zeneca as a candidate drug for the treatment of schizophrenia. In this case the final *ee* could be improved from 60% to 80% by optimising the Ti:tartrate ratio [304].

From a practical viewpoint the recently discovered vanadium-based and iron-based asymmetric sulfoxidation with hydrogen peroxide is worth mentioning [305, 306]. For vanadium, in principle as little as 0.01 mol% of catalyst can be employed (Fig. 4.111). With tridentate Schiff-bases as ligands, formed from readily available salicylaldehydes and (S)-*tert*-leucinol, *ee*s of 59–70% were obtained for thioanisole [305], 85% *ee* for 2-phenyl-1,3-dithiane [305] and 82–91% *ee* for *tert*-butyl disulfide [307]. For iron, similar results were obtained using 4 mol% of an iron catalyst, synthesized *in situ* from $Fe(acac)_3$ and the same type of Schiff base ligands as in Fig. 4.111 (see Ref. [306] for details).

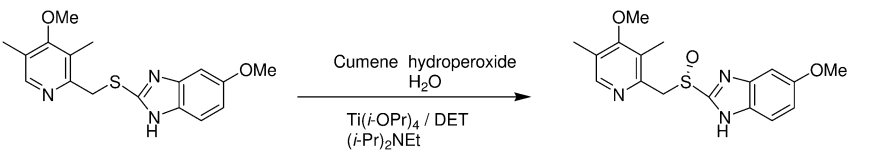


Fig. 4.110 Production of (S)-Omeprazole.

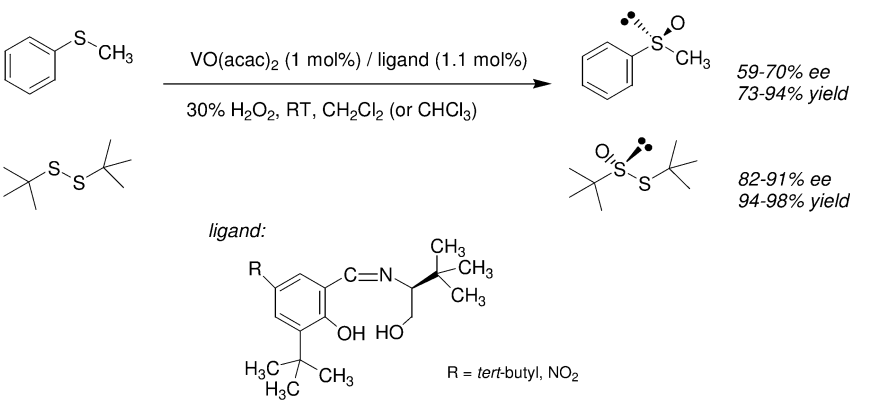


Fig. 4.111 Vanadium catalyzed asymmetric sulfoxidation.

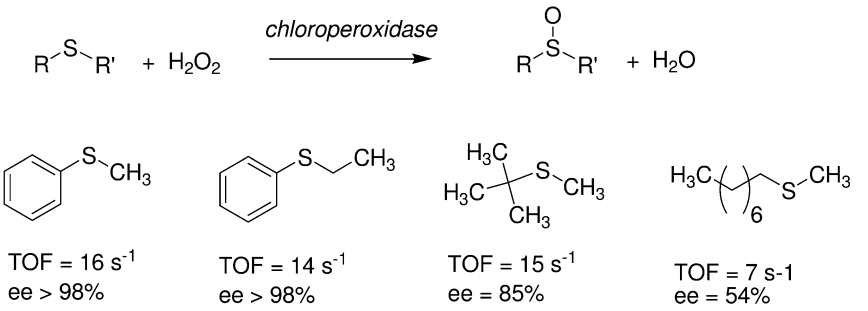


Fig. 4.112 Sulfoxidation reactions mediated by CPO.

Finally, but certainly not least, we note that the enzyme chloroperoxidase (CPO), catalyzes the highly enantioselective (>98% *ee*) sulfoxidation of a range of substituted thioanisoles [308]. In contrast to the epoxidation of alkenes, where turnover frequencies were low (see above), in the case of sulfoxidation of thioanisole a turnover frequency of around 16 s^{-1} and a total turnover number of 125 000 could be observed. A selection of data is represented in Fig. 4.112. Besides aryl alkyl sulfides, also dialkylsulfides could be oxidized with reasonable enantioselectivities [27].

Another class of peroxidases which can perform asymmetric sulfoxidations, and which have the advantage of inherently higher stabilities because of their non-heme nature, are the vanadium peroxidases. It was shown that vanadium bromoperoxidase from *Ascophyllum nodosum* mediates the production of (*R*)-methyl phenyl sulfoxide with a high 91% enantiomeric excess from the corresponding sulfide with H_2O_2 [38]. The turnover frequency of the reaction was found to be around 1 min^{-1}. In addition this enzyme was found to catalyse the sulfoxidation of racemic, non-aromatic cyclic thioethers with high kinetic resolution [309].

4.7.4
Asymmetric Baeyer-Villiger Oxidation

The area of asymmetric catalytic Baeyer-Villiger oxidation was recently reviewed [310]. It was concluded that biocatalytic methods in this case would seem to have the edge with respect to enantioselectivity, regiochemistry, and functional group selectivity. Baeyer-Villiger monooxgenases (BVMOs) are versatile biocatalysts that have been widely used in synthetic biotransformations. The cyclohexanone monooxygenase (CHMO) from *Acinetobacter calcoaceticus* NCIMB is the best characterized and most studied of these enzymes [311]. They contain a flavin moiety as the prosthetic group and utilize NAD(P)H as a stoichiometric cofactor (see Fig. 4.113).

In view of the requirement for cofactor regeneration these reactions are generally performed with whole microbial cells in a fermentation mode. Degrada-

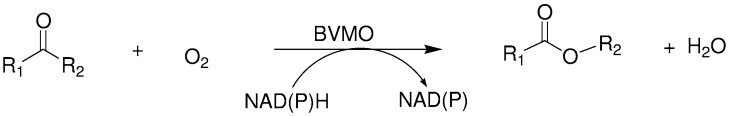


Fig. 4.113 BV reactions catalyzed by BVMOs.

tion of the lactone product can be circumvented by the addition of hydrolase inhibitors and/or heterologous expression. Several CHMOs are now commercially available, e.g. from Fluka and they are easy to use, even for non-specialists. A disadvantage of the methodology is the low volume yield (concentrations are typically 10 mM compared to 1 M solutions in homogeneous catalysis). Furthermore, many organic substrates have limited solubility in water. This problem could possibly be solved by performing the reactions in organic media [312]. The generally accepted mechanism for BV oxidations catalyzed by BVMOs was previously discussed in Section 4.2 (see Fig. 4.15). The state-of-the-art with regard to scale-up of BVMO catalyzed oxidations has been recently reviewed [313]. The reaction suffers from both substrate and product inhibition and the optimum ketone concentration was shown to be 0.2 to 0.4 g l^{-1} and at product concentrations above 4.5 to 5 g l^{-1} the activity of the whole cell biocatalyst fell to zero. This suggests that a fed-batch operation with continuous product removal, by adsorption on a solid resin, is necessary in order to obtain reasonable volumetric yields. In this way volumetric yields up to 20 g l^{-1} could be obtained.

In principle, the use of the isolated enzyme should allow the reaction to be performed at higher substrate concentrations, avoid side reactions and facilitate downstream processing. However, this requires an ancillary enzymatic cofactor regeneration system. Encouraging results have been obtained by coupling CHMO-catalyzed BV oxidation to cofactor regeneration mediated by an NADPH-dependent formate dehydrogenase (Fig. 4.114) [314, 315]. The overall reaction constitutes an enantioselective BV oxidation with stoichiometric consumption of O_2 and formate to give water and carbon dioxide as the coproducts. The reaction was carried to complete conversion with a 40 mM substrate concentration and the enzymes separated by ultrafiltration and re-used.

The enzyme catalyzed hydrolysis of the lactone and subsequent oxidation are unwanted side-reactions, obviously. Several yeasts [316] and *E. coli* [317] have

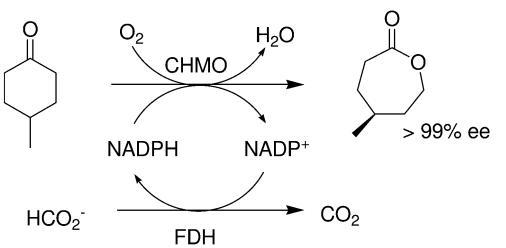


Fig. 4.114 CHMO catalyzed BV reaction coupled to cofactor regeneration by formate dehydrogenase.

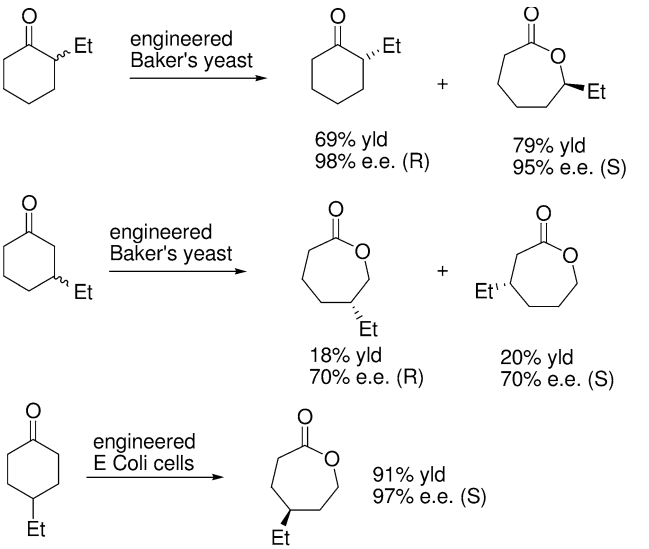


Fig. 4.115 Enzyme catalyzed asymmetric BV oxidation of 2-, 3- and 4-ethylcyclohexanones.

been engineered to convert the ketone to the lactone exclusively. Thus 2-substituted [316] and 4-substituted (or prochiral), [317, 318] cyclohexanones can be converted with high selectivity (Fig. 4.115) [319]. The (racemic) 3-substituted [320] cyclohexanones are synthetically less interesting as they can yield two regioisomeric lactones, each in two enantiomers. For small substituents there is little discrimination by the enzyme.

The first example of a dynamic kinetic resolution involving a CHMO-catalyzed BV oxidation was recently reported (Fig. 4.116) [321]. The reaction was performed with whole cells of CHMO-containing recombinant *E. coli* sp. at pH 9. Under these conditions the ketone substrate underwent facile racemization,

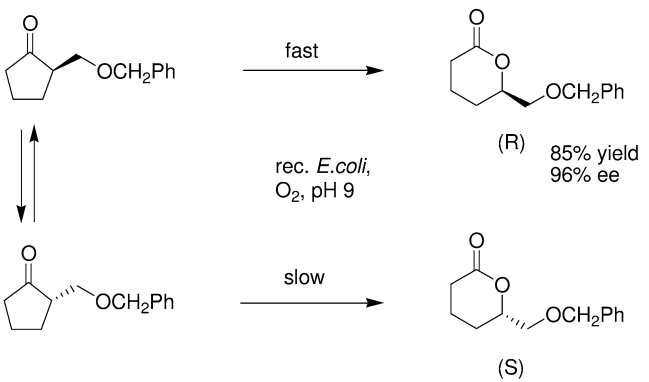


Fig. 4.116 Dynamic kinetic resolution of ketones using CHMO-containing recombinant *E. coli*.

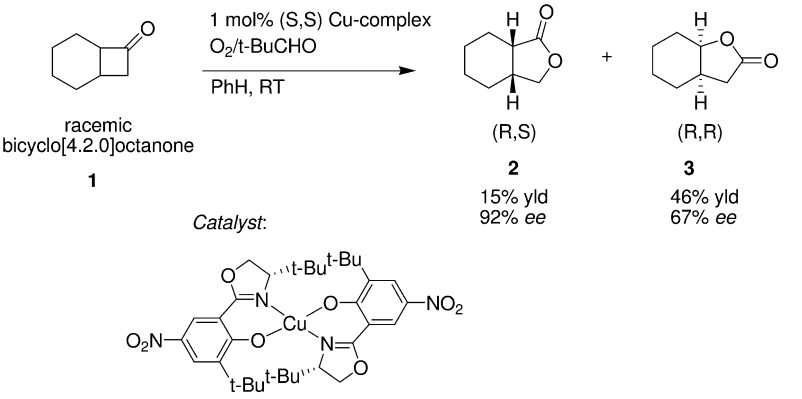


Fig. 4.117 Asymmetric copper-catalyzed BV oxidation of cyclobutanones.

via keto–enol tautomerism, and the lactone product was obtained in 85% yield and 96% *ee*.

Homogeneous catalysts can also be used for asymmetric BV oxidation [310]. One of the best catalysts appears to be 1 mol% of a chiral copper catalyst with oxazoline ligands (see Fig. 4.117), cyclobutanone derivatives are readily oxidized to give optically active lactones. The most promising results were obtained in reactions using benzene solutions under an atmosphere of dioxygen (1 atm) with pivaldehyde as co-reductant at ambient temperature [322]. In general, 2-aryl substituted cyclohexanones give good results. In the example of Fig. 4.117, metal catalyzed oxidation of rac-**1** gave isomeric γ-lactones **2** and **3** in a ratio of 3 : 1 (61% yield) with enantiomeric excesses of 67 and 92% respectively [323].

4.5 Conclusion

Summarizing, it is evident that catalytic oxidation is a mature technology which is still under strong development. Every day the performance of catalysts that use O_2 or H_2O_2 as green oxidants, increases. This is illustrated by two key areas, the oxidation of alcohols and alkenes. In the first case, the oxidation of alcohols no longer requires stoichiometric use of heavily polluting metals. Many homogeneous and heterogeneous catalysts are available which use molecular oxygen as the oxidant. Especially promising seems to be the use of gold nanoparticles as catalysts for alcohol and carbohydrate oxidation. The turnover frequencies obtained in this case overwhelm all results obtained so far. In the area of asymmetric epoxidation, the use of hydrogen peroxide now seems to give as good results as the more established methods using alkylhydroperoxide or hypochlorite as the oxidant.

When comparing homogeneous and heterogeneous catalysts, clearly homogeneous systems take the lead. In addition, heterogeneous systems have the disad-

vantage that the leaching of metals in solution is always a possible complication in the reaction, and therefore they have to be subjected to a rigorous test of heterogeneity.

When comparing chemical and biocatalytic methods, one could say that, especially for asymmetric oxidations, enzymatic methods enter the scene. This is most evident in the area of asymmetric Baeyer-Villiger oxidation, where biocatalysts take the lead and homogeneous chiral catalysts lag far behind in terms of *ee* values. Significant progress can be expected in the area of biocatalysis due to the advancement in enzyme production technologies and the possibility of tailor-made enzymes.

Despite the huge effort put into research towards green and catalytic oxidation, the number of applications in the fine chemical industry is limited. For industrial batch processes simple, robust and reliable methods are required. For oxidations, this is in general less straightforward than for reductions. However, much is to be gained in this area, and therefore elegance should be a leading principle for all newly designed chemical oxidation processes.

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5
Catalytic Carbon–Carbon Bond Formation

5.1
Introduction

The formation of carbon–carbon bonds is central to organic chemistry, indeed to chemistry in general. The preparation of virtually every product, be it fine chemical or bulk chemical, will include a carbon–carbon bond formation at some stage in its synthesis. The importance of carbon–carbon bond forming reactions can therefore not be overemphasized. Consequently they have been a focus of interest ever since chemists made their first attempts at synthesis. In the course of the last 150 years many very selective and efficient reactions for the formation of carbon–carbon bonds have been introduced, too many for the scope of this book. At the same time carbon–carbon bond forming reactions are often textbook examples of wastefulness. The Nobel prize winning Wittig reaction being a particularly good illustration of why green chemistry is necessary [1]. At the same time it also becomes obvious how much there still remains to be done before chemistry is green, since not even the equally Nobel prize winning metathesis can completely replace the Wittig reaction.

This chapter focuses on the application of transition metal catalysts and enzymes for the formation of carbon–carbon bonds. Transition metal-catalyzed carbon–carbon bond formations are not always very green but they often replace even less favorable conventional approaches. The key to making them really green is that they have to be easily separable and reusable. Several of these reactions, such as the hydroformylation, oligomerisation, carbonylation of alcohols and the metathesis, are therefore also treated in Chapter 1, Section 1.8 and Chapter 7, since their greener variations are performed in novel reaction media.

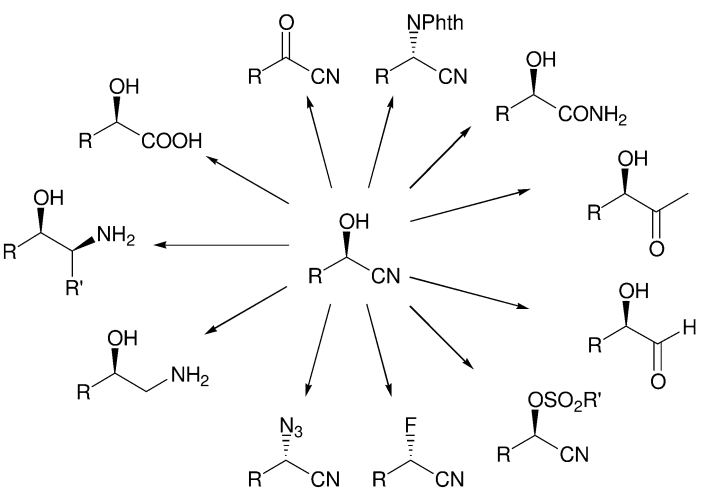
5.2
Enzymes for Carbon–Carbon Bond Formation

The synthesis of carbon–carbon bonds in nature is performed by a vast variety of enzymes, however the bulk of the reactions are performed by a rather limited number of them. Indeed, for the synthesis of fatty acids just one carbon–carbon bond forming enzyme is necessary. Contrary to expectation, virtually none of

the enzymes that nature designed for building up molecules are used in organic synthesis. This is because they tend to be very specific for their substrate and can therefore not be broadly applied. Fortunately an ever increasing number of enzymes for the formation of carbon–carbon bonds are available [2–4]. Many of them are lyases and, in an indirect approach, hydrolases. These classes of enzymes were designed by nature not for making but for breaking down molecules. By reversing the equilibrium reactions that these enzymes catalyze in the natural substrates they can be applied to the synthesis of carbon–carbon bonds. As reagents that were designed by nature for degrading certain functional groups, they are robust but not very substrate specific. However, they are specific for the functional group they destroy in nature and generate in the laboratory or factory. Equally important, they are very stereoselective. Hence these enzymes are ideal for the application in organic synthesis.

5.2.1
Enzymatic Synthesis of Cyanohydrins

Cyanohydrins are versatile building blocks that are used in both the pharmaceutical and agrochemical industries [2–9]. Consequently their enantioselective synthesis has attracted considerable attention (Scheme 5.1). Their preparation by the addition of HCN to an aldehyde or a ketone is 100% atom efficient. It is, however, an equilibrium reaction. The racemic addition of HCN is base-catalyzed, thus the enantioselective, enzymatic cyanide addition should be performed under mildly acidic conditions to suppress the undesired background reaction. While the formation of cyanohydrins from aldehydes proceeds readily, the equilibrium for ketones lies on the side of the starting materials. The latter reaction can therefore only be performed successfully by either bio- or chemo-cat-



Scheme 5.1 Cyanohydrins are versatile building blocks.

alysis when an excess of HCN is employed, or when the product is constantly removed from the equilibrium. The only advantage of this unfavorable equilibrium is that the liquid acetone cyanohydrin can replace the volatile HCN in the laboratory. It releases HCN during the synthesis of an aldehyde-based cyanohydrin, improving safety in the laboratory significantly. At the same time the atom efficiency of the reaction is, however, greatly reduced. Alternative methods for the safe handling of cyanides on a laboratory scale are for instance to use cyanide salts in solution, again generating waste. In order to achieve high yields an excess of HCN, or of the other cyanide sources, is commonly used. This excess cyanide needs to be destroyed with iron (II) sulfate or bleach in the laboratory. It is much easier to handle HCN on an industrial scale and to regain any HCN. In general all work involving cyanide (enzyme-catalyzed or not) must be performed in well-ventilated laboratories and HCN detectors have to be used [6].

5.2.1.1 **Hydroxynitrile Lyases**

For the synthesis of cyanohydrins nature provides the chemist with *R*- and *S*-selective enzymes, the hydroxynitrile lyases (HNL) [4–7]. These HNLs are also known as oxynitrilases and their natural function is to catalyze the release of HCN from natural cyanohydrins like mandelonitrile and acetone cyanohydrin. This is a defense reaction of many plants. It occurs if a predator injures the plant cell. The reaction also takes place when we eat almonds. Ironically the benzaldhyde released together with the HCN from the almonds is actually the flavor that attracts us to eat them.

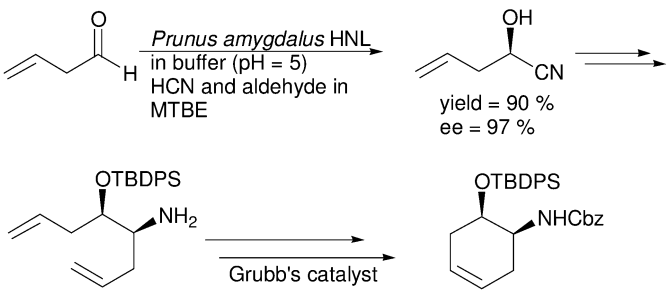
Since the release of HCN is a common defense mechanism for plants, the number of available HNLs is large. Depending on the plant family they are isolated from, they can have very different structures; some resemble hydrolases or carboxypeptidase, while others evolved from oxidoreductases. Although many of the HNLs are not structurally related they all utilize acid–base catalysis. No co-factors need to be added to the reactions nor do any of the HNL metallo-enzymes require metal salts. A further advantage is that many different enzymes are available, *R*- or *S*-selective [10]. For virtually every application it is possible to find a stereoselective HNL (Table 5.1). In addition they tend to be stable and can be used in organic solvents or two-phase systems, in particular in emulsions.

The *R*-selective *Prunus amygdalus* HNL is readily available from almonds. Approximately 5 g of pure enzyme can be isolated from 1 kg of almonds, alternatively crude defatted almond meal has also been used with great success. This enzyme has already been used for almost 100 years and it has successfully been employed for the synthesis of both aromatic and aliphatic *R*-cyanohydrins (Scheme 5.2) [11]. More recently it has been cloned into *Pichea pastoris*, guaranteeing unlimited access to it and enabling genetic modifications of this versatile enzyme [12].

Prunus amygdalus HNL can be employed for the bulk production of (*R*)-*o*-chloromandelonitrile, however with a modest enantioselectivity (*ee*=83%) [9]. When replacing alanine 111 with glycine the mutant HNL showed a remarkably

Table 5.1 Commonly used hydroxynitrile lyases (HNL).

Name and origin	Natural substrate	Stereoselectivity
Prunus amygdalus HNL, Almonds	(*R*)-mandelonitrile	*R*
Linum usitatissimum HNL, Flax seedlings	(*R*)-butanone cyanohydrin and acetone canohydrin	*R*
Hevea brasiliensis HNL, Rubber-tree leaves	acetone cyanohydrin	*S*
Sorghum bicolor HNL, Millet seedlings	(*S*)-4-hydroxy-mandelonitrile	*S*
Manihot esculenta HNL, Manioc leaves	acetone cyanohydrin	*S*



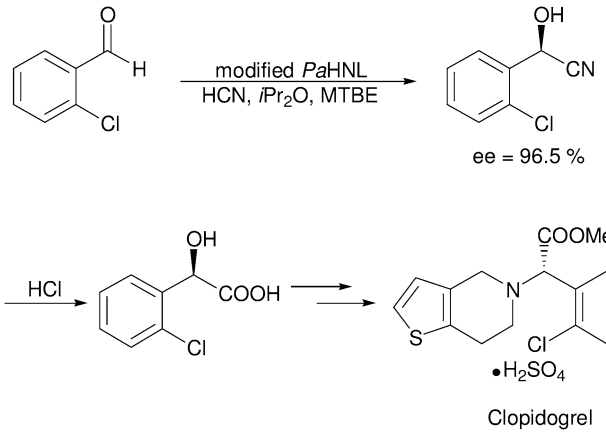
Scheme 5.2 Application of *Prunus amygdalus* HNL for the synthesis of *R*-cyanohydrins.

high enantioselectivity towards *o*-chlorobenzaldehyde and the corresponding cyanohydrin was obtained with an *ee* of 96.5% [12]. Hydrolysis with conc. HCl yields the enantiopure (*S*)-*o*-chloromandelic acid, an intermediate for the antithrombotic drug clopidogrel (Scheme 5.3).

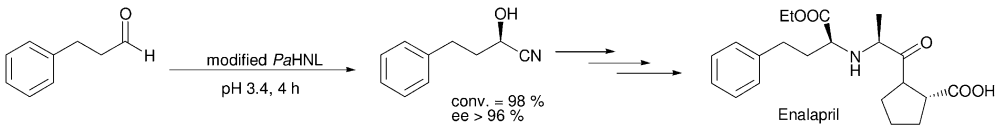
Recently it was described that site directed mutagenesis has led to a *Prunus amygdalus* HNL that can be employed for the preparation of (*R*)-2-hydroxy-4-phenylbutyronitrile with excellent enantioselectivity (*ee* > 96%). This is a chiral building block for the enantioselective synthesis of ACE inhibitors such as enalapril (Scheme 5.4) [13].

The unmodified enzyme has also been used in ionic liquids, expanding the scope of its application even further. Significant rate enhancement, in particular for substrates that otherwise react only sluggishly, was observed [14].

The discovery of the *S*-selective HNLs is more recent. The application of the *S*-selective *Hevea brasiliensis* HNL was first described in 1993 [15]. The potential of this enzyme was immediately recognized and already four years after describing it for



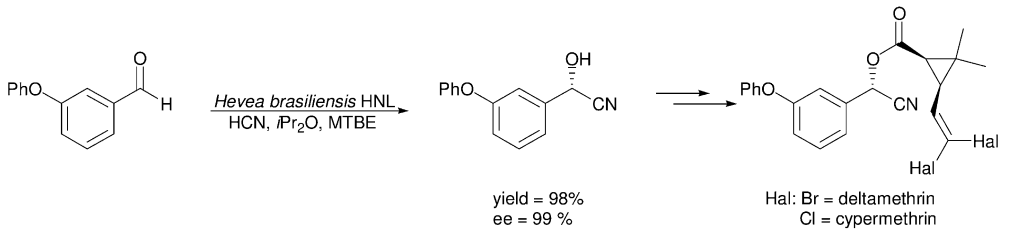
Scheme 5.3 Modified *Prunus amygdalus* HNL is applied for the synthesis of enantiopure *R*-*o*-chloromandelonitrile, a precursor for the synthesis of clopidogrel.



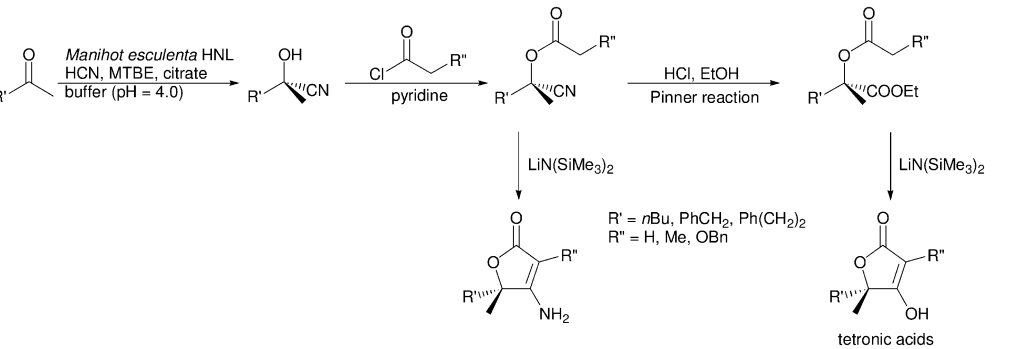
Scheme 5.4 Modified *Prunus amygdalus* HNL catalyzes the enantioselective formation of potential precursors for ACE inhibitors.

the first time it has been cloned and overexpressed, making it available for large-scale applications [16]. Indeed, this enzyme is not only a versatile catalyst in the laboratory, industrially it is used to prepare (*S*)-*m*-phenoxymandelonitrile with high enantiopurity (*ee* > 98%) [9]. This is a building block for the pyrethroid insecticides deltamethrin and cypermethrin and is used on a multi-ton scale (Scheme 5.5). The HNL-based process replaced another enzymatic process that was used earlier for the production of the enantiopure cyanohydrin, the kinetic resolution of *m*-phenoxymandelonitrile acetate with a hydrolase from *Arthrobacter globiformis* [17].

As mentioned above, the synthesis of cyanohydrins from ketones is difficult due to the unfavorable equilibrium. However, it can be achieved. When methyl ketones were treated with only 1.5 equivalents of HCN in an emulsion of citrate buffer (pH 4.0) and MTBE, the *S*-selective *Manihot esculenta* HNL catalyzed the synthesis of the corresponding cyanohydrins with good yields (85–97%) and enantioselectivities (69–98%). The corresponding esters were then used in a second carbon–carbon bond forming reaction. Since cyanohydrins from ketones cannot be deprotonated adjacent to the nitrile group, the esters could be selectively deprotonated and then a ring closing attack on the nitrile function yielded the unsaturated lactones (Scheme 5.6). When the nitrile function was first con-



Scheme 5.5 *Hevea brasiliensis* HNL catalyzes a key step in the enantioselective synthesis of pyrethroid insecticides.



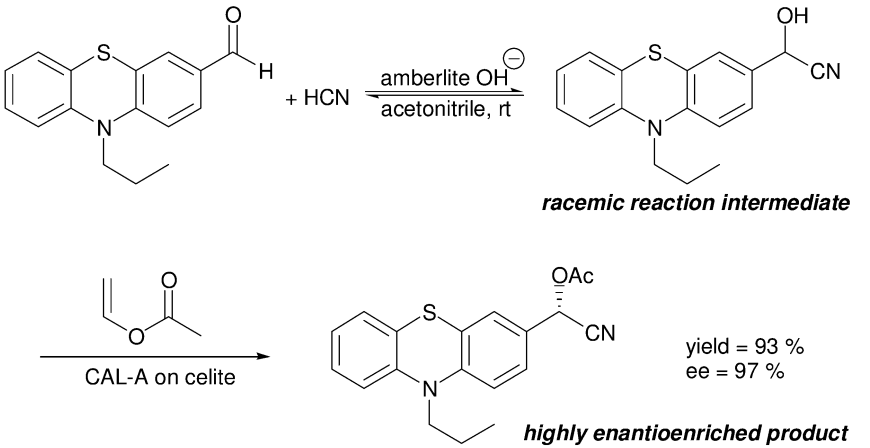
Scheme 5.6 HNL-catalyzed formation of cyanohydrins from ketones and their application in synthesis.

verted into an ester via a Pinner reaction, the intramolecular Claisen reaction gave chiral tetronic acids [18].

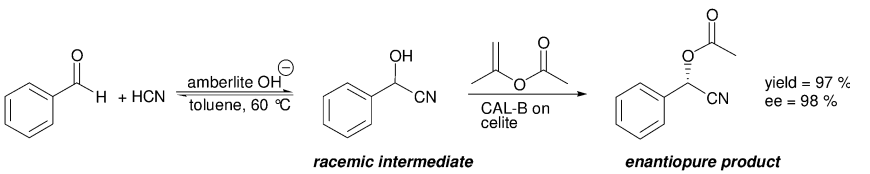
5.2.1.2 **Lipase-based Dynamic Kinetic Resolution**

A completely different enzyme-catalyzed synthesis of cyanohydrins is the lipase-catalyzed dynamic kinetic resolution (see also Chapter 6). The normally undesired, racemic base-catalyzed cyanohydrin formation is used to establish a dynamic equilibrium. This is combined with an irreversible enantioselective kinetic resolution via acylation. For the acylation, lipases are the catalysts of choice. The overall combination of a dynamic carbon–carbon bond forming equilibrium and a kinetic resolution in one pot gives the desired cyanohydrins protected as esters with 100% yield [19–22].

This methodology was employed to prepare many heterocyclic cyanohydrin acetates in high yields and with excellent enantioselectivities, *Candida antarctica* lipase A (CAL-A) being the lipase of choice (Scheme 5.7) [23]. A recent detailed study of the reaction conditions revealed that the carrier on which the lipase is immobilised is important; generally Celite should be used for aromatic substrates. With Celite R-633 as support for *Candida antarctica* lipase B (CAL-B)



Scheme 5.7 CAL-A-catalyzed formation of chiral cyanohydrins.



Scheme 5.8 Synthesis of enantiopure mandelonitrile acetate via a dynamic kinetic resolution.

mandelonitrile acetate was synthesised in 97% yield and *ee*=98% (Scheme 5.8) [24, 25]. Drawbacks of this approach are that an *R*-selective variant has so far not been developed and most lipases do not accept sterically demanding acids. Consequently, attempts at preparing the pyrethroid insecticides in one step via this approach were not successful.

5.2.2
Enzymatic Synthesis of α-Hydroxyketones (Acyloins)

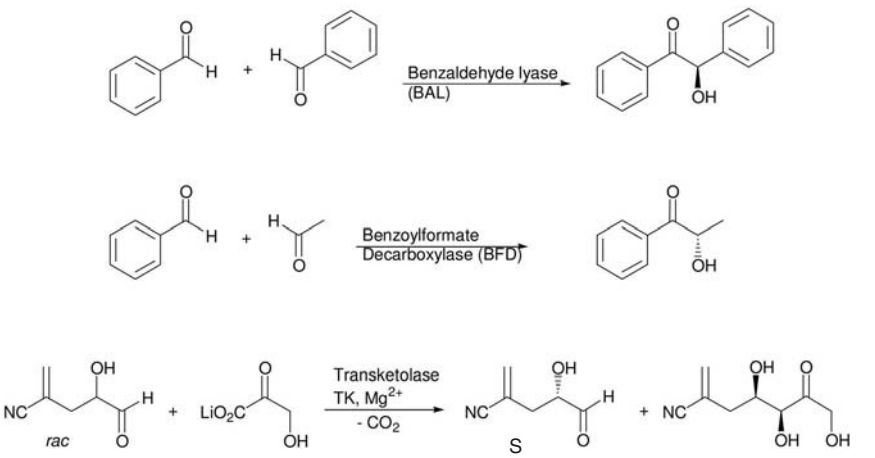
The bifunctional nature and the presence of a stereocenter make α-hydroxyketones (acyloins) amenable to further synthetic transformations. There are two classical chemical syntheses for these α-hydroxyketones: the acyloin condensation and the benzoin condensation. In the acyloin condensation a new carbon–carbon bond is formed by a reduction, for instance with sodium. In the benzoin condensation the new carbon–carbon bond is formed with the help of an umpolung, induced by the formation of a cyanohydrin. A number of enzymes catalyze this type of reaction, and as might be expected, the reaction conditions are considerably milder [2–4, 26, 27]. In addition the enzymes such as benzaldehyde lyase (BAL) catalyze the formation of a new carbon–carbon bond enantioselectively. Transketolases (TK)

and decarboxylases, such as benzoylformate decarboxylase (BFD), catalyze the acyloin formation efficiently, too. Like the chemical benzoin reaction the latter two break a carbon–carbon bond while forming a new one. This making and breaking of bonds involves a decarboxylation in both the TK and the decarboxylase-catalyzed reactions. When TK is applied it might also involve the discarding of a ketone and not only CO_2 [27, 28]. The atom efficiency of these enzymes is therefore limited. In contrast to TK, BFD can, however, also catalyze the formation of new carbon–carbon bonds without decarboxylation (Scheme 5.9).

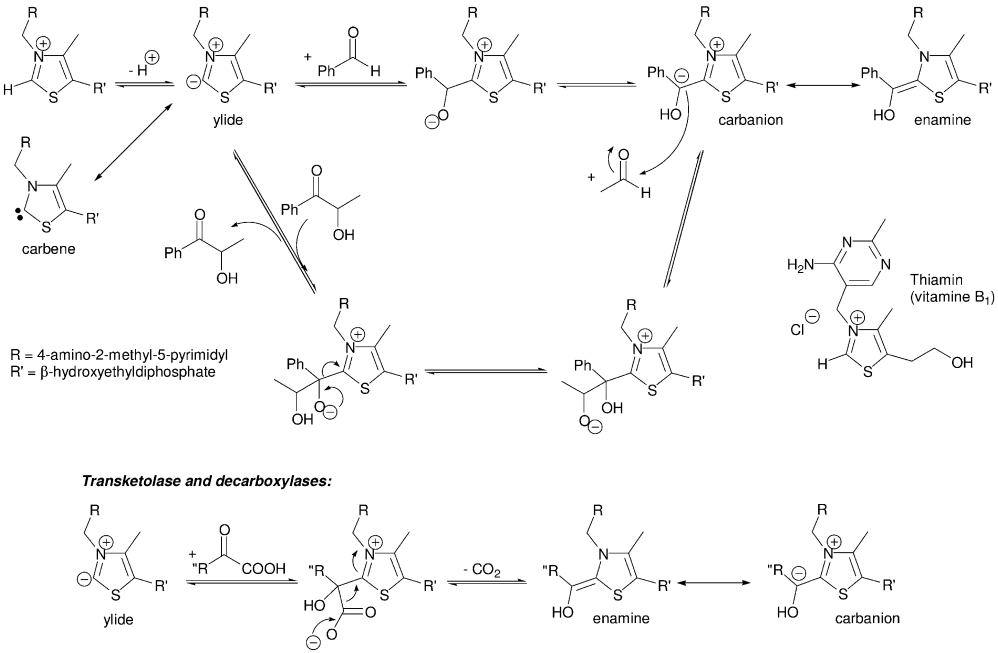
All of the enzymes that catalyze the acyloin formation from two carbonyl groups rely on a cofactor: thiamin diphosphate. The unphosphorylated thiamin is also known as vitamin B1, deficiency thereof is the cause of the disease Beriberi. Thiamin diphosphate induces an umpolung of the carbonyl group, similar to the cyanide in the benzoin condensation. After deprotonation its ylide attacks the carbonyl group of the aldehyde. Shifting of the negative charge from the alcoholate to the carbanion initiates this umpolung. This enables the nucleophilic attack on the second aldehyde, stereoselectively forming the carbon–carbon bond for the α-hydroxyketones. Another shift of the charge and elimination releases the desired acyloin and the ylide is available for further action (Scheme 5.10).

In the decarboxylase- and TK-catalyzed reactions the ylide attacks the keto function of α-keto acids to form an intermediate carboxylate ion. Decarboxylation, i.e. carbon–carbon bond fragmentation leads to the same reactive carbanion as in the BAL-catalyzed reaction (Scheme 5.10). Once again, it enantioselectively forms the new carbon–carbon bond of the desired α-hydroxyketone. TK requires not only thiamin diphosphate but also a second co-factor, Mg^{2+} [28].

The enantioselective synthesis of α-hydroxyketones via a carbon–carbon bond forming reaction has received a significant impulse during recent years. Four different enzymes are commonly used for this reaction: BAL, BFD, pyruvate decarboxylase (PDC) and TK. Many different compounds can be prepared with



Scheme 5.9 BAL, BFD and TK catalyze the formation of α-hydroxyketones (acyloins).

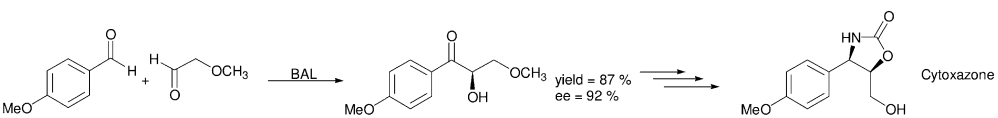


Scheme 5.10 Catalytic cycle in thiamin diphosphate based carbon–carbon bond forming enzymes.

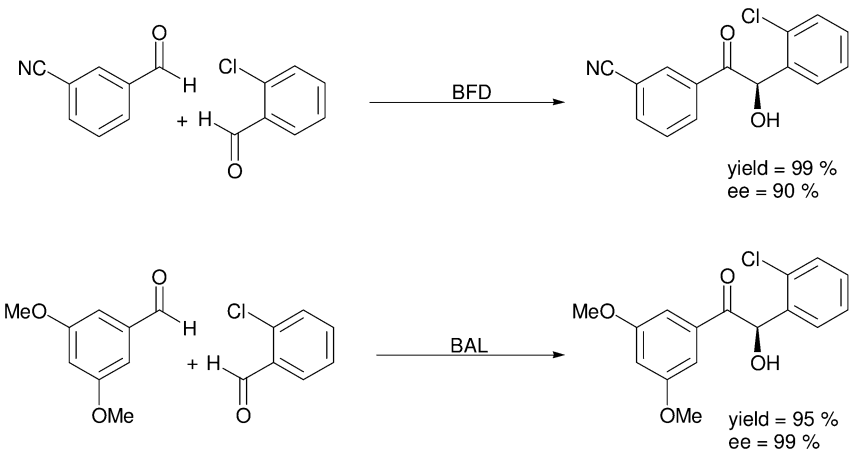
their aid, however, not every stereoisomer is accessible yet. Moreover, it is still difficult to perform the reaction of two different aldehydes to obtain the mixed acyloins in a predictable manner.

The *R*-selective BAL accepts either two aromatic aldehydes or an aliphatic and an aromatic aldehyde as substrates. This enables enantioselective access to a variety of biologically active compounds and natural products such as Cytoxazone, a metabolite from *Streptomyces sp.* (Scheme 5.11) [29]. If formaldehyde is used instead of prochiral acetaldehyde or its derivatives achiral hydroxyacetophenones are obtained [30].

Both BAL and BFD can be applied for *R*-selective cross coupling reactions between two aromatic aldehydes (Scheme 5.12). This opens a direct access to a huge variety of compounds. Their stereoselectivity in these reactions is identical, their substrate specificity however, is not. Of particular interest is that BFD is only *R*-selective for the coupling of two aromatic aldehydes. For the coupling of



Scheme 5.11 BAL-catalyzed synthesis of carbon–carbon bonds.

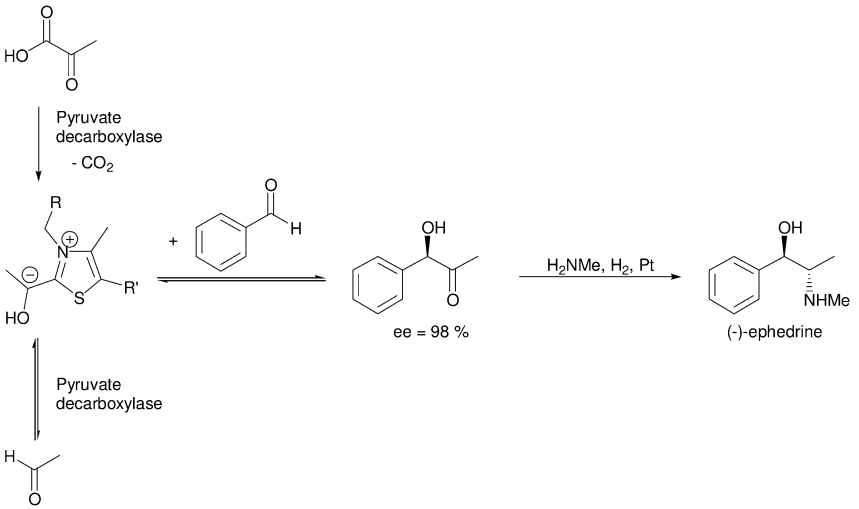


Scheme 5.12 BAL and BFD-catalyzed cross coupling reactions between two aromatic aldehydes.

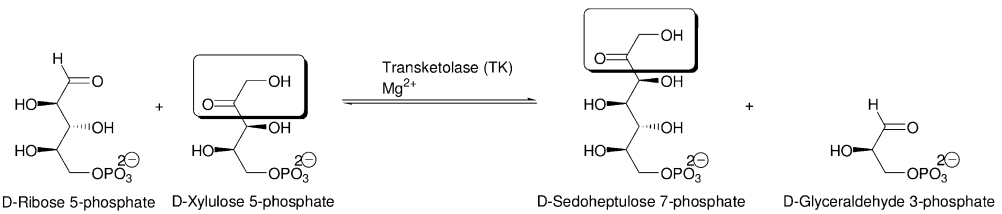
two aliphatic aldehydes or of aliphatic aldehydes with aromatic aldehydes BFD is *S*-selective (Schemes 5.9 and 5.12). Consequently BAL and BFD complement each other's stereoselectivity in coupling reactions between an aromatic and aliphatic aldehyde, widening the scope of the cross coupling approach [31, 32].

In the above-described cross coupling reactions between an aromatic and an aliphatic aldehyde the keto group of the product is always adjacent to the benzene ring (Schemes 5.9 and 5.12). With pyruvate decarboxylase (PDC) this selectivity is turned around. It catalyzes the reaction of pyruvate with benzaldehyde. After decarboxylation of the pyruvate a new carbon–carbon bond is formed. Fortunately acetaldehyde can be used instead of pyruvate, improving the atom efficiency of the reaction. With either pyruvate or acetaldehyde the cross coupling with benzaldehyde yields (*R*)-phenylacetylcarbinol in good optical purity. This process has been performed on an industrial scale since the 1930s. Whole yeast cells are used and pyruvate is employed as a starting material, together with benzaldehyde. This acyloin is converted via a platinum-catalyzed reductive amination on a commercial scale into (–)-ephedrine (Scheme 5.13) [26, 33]. The newest development for the industrial application is the introduction of the PDC from *Zymomonas mobilis*, a bacterial PDC [33, 34]. This PDC accepts acetaldehyde instead of the significantly more expensive pyruvate. While the industrial synthesis of (*R*)-phenylacetylcarbinol with PDC was very successful, PDC has not been used for many other acyloin syntheses.

The sugar metabolism is a source of many enzymes, the transketolase (TK) being one of them. TK transfers an *α*-hydroxy carbonyl fragment from D-xylulose-5-phosphate onto D-ribose-5-phosphate, forming D-sedoheptulose-7-phosphate and D-glyceraldehyde-3-phosphate (Scheme 5.14). Since this reaction is an equilibrium reaction and starting materials and products are of similar stability, it is not very versatile for organic synthesis. Fortunately TK also accepts pyruvate instead of xylulose. Under these modified circumstances carbon dioxide

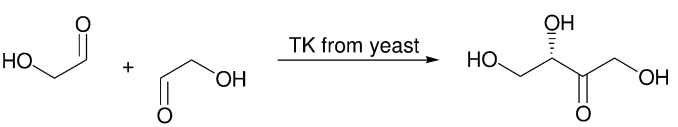


Scheme 5.13 The PDC-catalyzed synthesis of (*R*)-phenylacetylcarbinol induces the stereochemistry in the industrial synthesis of (–)-ephedrine.



Scheme 5.14 In nature TK catalyzes an equilibrium reaction.

and not D-glyceraldehyde-3-phosphate is the leaving group. This renders the reaction irreversible and considerably more atom efficient [28]. More interesting still is the recent observation that yeast TK can activate the two-carbon unit glycolaldehyde. No decarboxylation occurs and the atom efficiency of this coupling reaction yielding erythrulose (Scheme 5.15) is 100% [35]. This result demonstrated that TK can be employed similar to an aldolase and the path is now open for efficient cross coupling reactions.



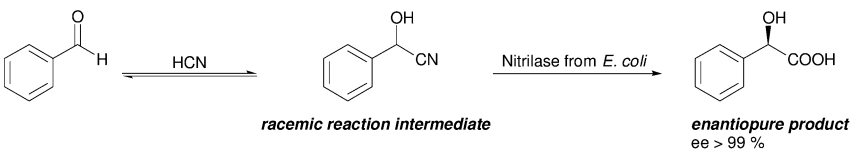
Scheme 5.15 TK can be employed like an aldolase in a 100% atom efficient reaction.

5.2.3
Enzymatic Synthesis of α-Hydroxy Acids

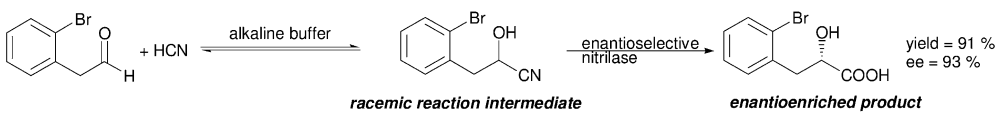
α-Hydroxy acids are valuable building blocks and can be prepared by the chemical hydrolysis of enantiopure cyanohydrins, as described above. Utilising a dynamic kinetic resolution, it is, however, possible to prepare them from aldehydes in a single step. This is achieved by combining the base-catalyzed equilibrium between the aldehyde and HCN with a racemic cyanohydrin with an irreversible, enantioselective follow up reaction (see Section 5.2.1.2). The *in situ* hydrolysis of just one enantiomer of the cyanohydrin, catalyzed by an enantioselective nitrilase [36, 37], yields the desired α-hydroxy acids in excellent enantiopurity. Thus the actual carbon–carbon bond formation is not catalyzed by the enzyme, the nitrilase does, however, impart the enantioselectivity.

Nature provides a vast variety of *R*-selective nitrilases that accept aliphatic and aromatic substrates. This disconnection can therefore be applied for the synthesis of many structurally different α-hydroxy acids. Industrially the process is applied on a multi-ton scale, to prepare (*R*)-mandelic acid and its analogs (Scheme 5.16) [9]. What makes the process particularly interesting from a green point of view, is that no organic solvents are used and the reaction is performed at ambient temperatures.

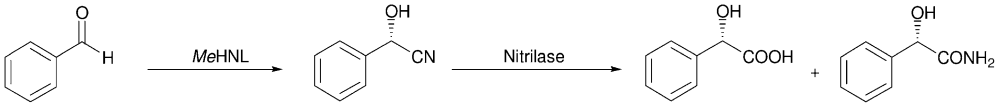
A drawback of this reaction has recently been addressed. Only very few *S*-selective nitrilases were known; this problem has been solved: a systematic screening program yielded a number of *S*-selective nitrilases that have successfully been employed in this dynamic kinetic resolution (Scheme 5.17) [38]. In an alternative approach, combining the enantioselectivity of an HNL with the hydrolytic power of a not very selective nitrilase that did accept cyanohydrins as substrates, the synthesis of optically enriched α-hydroxy acids starting from alde-



Scheme 5.16 The industrial synthesis of *R*-mandelic acid proceeds via a base and nitrilase-catalyzed dynamic kinetic resolution.



Scheme 5.17 *S*-α-hydroxy acids are prepared in high yield and optical purity via the dynamic kinetic resolution.



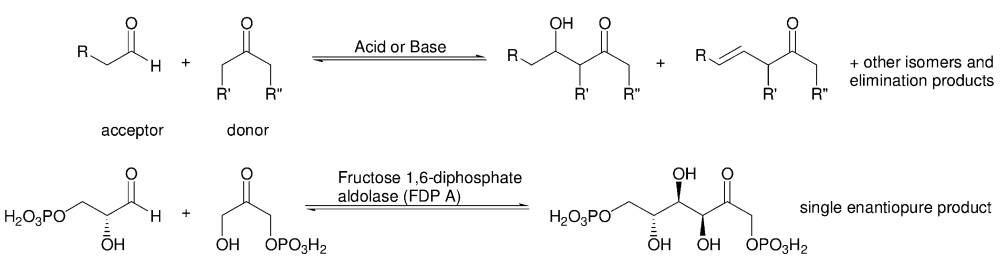
Scheme 5.18 Combining a HNL with an unselective nitrilase allows the synthesis of α-hydroxy acids.

hydes succeeded (Scheme 5.18). Surprisingly not only the desired acid was formed but the amide was also obtained [39].

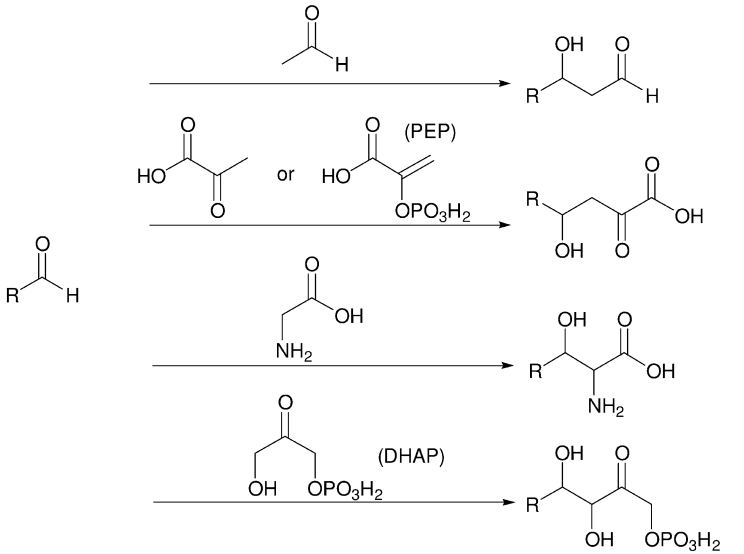
To date, this reaction cascade has no direct enantioselective chemo-catalytic equivalent. It is therefore a welcome addition to synthetic organic chemistry. Moreover, nitrile hydratases can potentially be used in this reaction, too, expanding its scope even further.

5.2.4
Enzymatic Synthesis of Aldols (β-Hydroxy Carbonyl Compounds)

The aldol reaction is one of the most versatile reactions for the formation of a new carbon–carbon bond and it is also highly atom efficient. Since its first description more than 120 years ago it has been the basis of the 1,3-disconnection in retrosynthesis. In contrast to the cyanohydrin synthesis there is a significant difference between the chemically catalyzed and the enzyme-catalyzed aldol reaction. The acid or base catalyzed aldol reaction is performed with starting materials that tend to have no functional groups in the α-position. The product is a β-hydroxy carbonyl compound, with the classical 1,3-functionality (Scheme 5.19). In the case of a cross coupling, care has to be taken to direct the reaction in such a manner that only one product is obtained, i.e. that only one molecule acts as a donor and the other as an acceptor. Moreover, there is always the risk that elimination will take place. The aldolases that catalyze the aldol reaction in nature are, similar to the TK, often obtained from the sugar metabolism. Consequently they convert highly substituted substrates and the products often have functional groups in the 1-, 2-, 3-, and 4-positions. Unlike the chemical reaction, the enzyme controls the cross coupling reactions very well and they almost al-



Scheme 5.19 Comparison of the chemically catalyzed aldol reaction with an aldolase-catalyzed reaction.



Scheme 5.20 Aldolases are classified according to the donor molecule they activate: DHAP, pyruvate, glycine and acetaldehyde.

ways yield just one product (Scheme 5.19), i.e. they display an excellent control over which molecule is the acceptor and which the donor. This has, however, one drawback: only a very limited number of carbonyl compounds can be used as donors [40]. Of course, the chemical reaction has successfully been converted into a selective reaction; proline and its derivates act as catalysts, which can be used directly with aldehydes and ketones [41]. In many cases, however, chiral auxiliaries in combination with highly reactive and toxic Lewis acids are employed, introducing extra steps and generating much waste [40, 42].

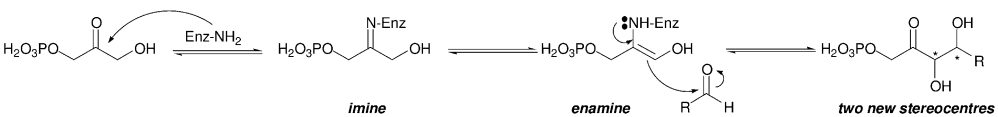
From a synthetic point of view, the chemically catalyzed and the aldolase-catalyzed reactions complement each other. Both can catalyze the synthesis of compounds that are difficult to obtain with the other type of catalyst. Aldolases have an excellent control over the regiochemistry and accept a wide variety of acceptor molecules. As mentioned above they allow only a few donor molecules. The aldolases that are commonly used activate four different donor molecules and are classified according to them (Scheme 5.20) [2–4, 40, 43]. Other aldolases are known, too, but their application for synthesis has so far been very limited and they will therefore not be discussed here.

5.2.4.1 **DHAP-dependent Aldolases**

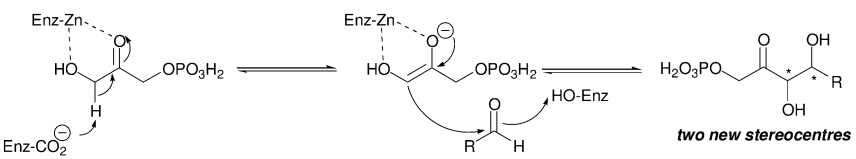
Dihydroxyacetone phosphate (DHAP) is the donor ketone that is utilized by the DHAP-dependent aldolases. These aldolases come under the class of lyases, just like the hydroxynitrile lyases (see Section 5.2.1.1). As for the HNLs, no cofactor

needs to be added to the reaction mixture and the DHAP-dependent aldolases are straightforward to use. There are two types of these aldolases: Type I aldolases, which are primarily found in higher plants and animals. They work by an enamine mechanism: the amino group of the active site lysine residue forms a Schiff's base with the carbonyl group of the donor DHAP and this activates the DHAP. The imine thus formed tautomerises to the enamine and this adds stereoselectively to the acceptor molecule (Scheme 5.21). Type II aldolases are found in fungi and bacteria; they contain a Zn^{2+} ion in the active site of the enzyme. This is thought to induce Lewis acidity in the enzyme. The zinc polarises the carbonyl group of the donor and forms the reactive enediolate. This nucleophile then adds to the acceptor aldehyde stereoselectively. A glutamate residue (Enz-COO-) and a tyrosine residue (Enz-OH) present in the active site of the enzyme assist in the removal and donation of protons respectively (Scheme 5.22). Both types of aldolases control the stereochemistry of the reaction, it is in most cases independent of the structure of the acceptor and many different acceptors can be employed with a high predictability to the stereochemical outcome of the synthesis. It is important to mention that both, Type I and Type II aldolases catalyze the same reactions, the formation of an aldol from DHAP and an acceptor aldehyde. Although they have completely different modes of action and are obtained from different organisms [40, 43]. This is similar to the HNLs: they too can be structurally very different while they all catalyze the formation of cyanohydrins [10].

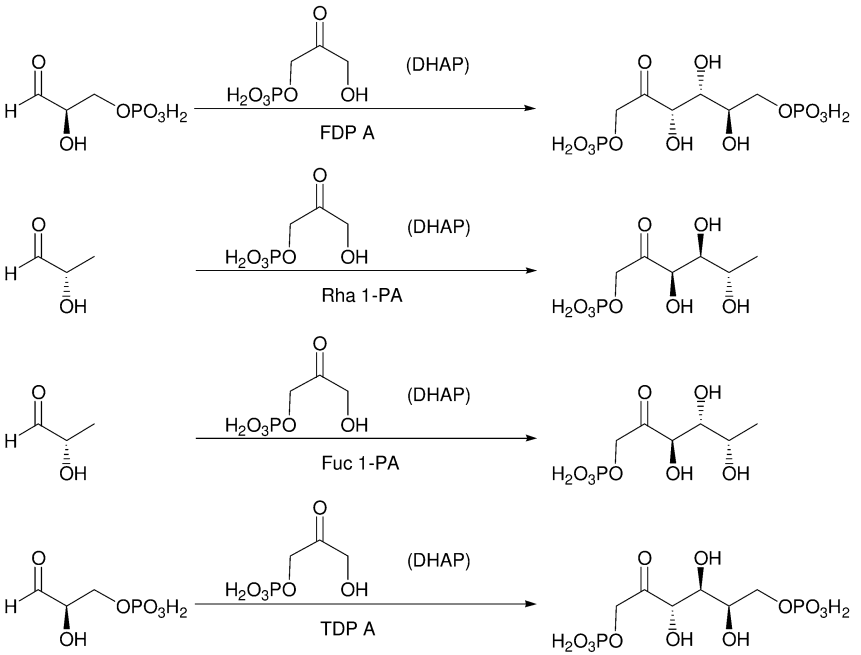
Two new stereocenters are established in the DHAP-dependent aldolases-catalyzed carbon–carbon bond formation. Consequently four different stereoisomers can be formed (Scheme 5.23). Enantioselective aldolases that catalyze the formation of just one of each of the stereoisomers are available: fructose 1,6-diphosphate aldolase (FDP A), rhamnulose 1-phosphate aldolase (Rha 1-PA), L-fuculose 1-phosphate aldolase (Fuc 1-PA) and tagatose 1,6-diphosphate aldolase (TDP A). In particular the FDP A, that catalyzes the formation of the D-threo stereochemistry, has been employed in many syntheses. One such FDP A that



Scheme 5.21 Reaction mechanism of a Type I aldolase.



Scheme 5.22 Reaction mechanism of a Type II aldolase.

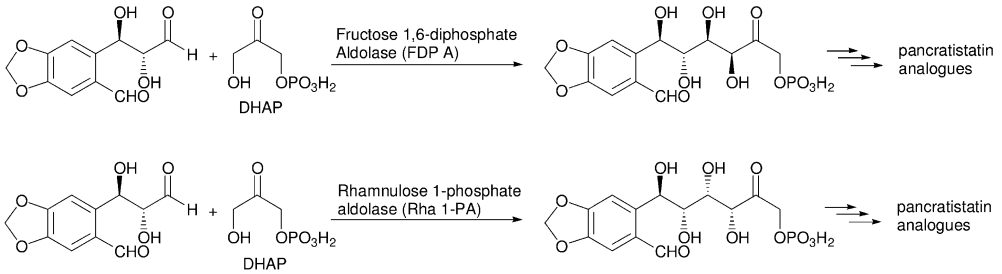


Scheme 5.23 The four different stereoisomers that can be formed are synthesized selectively by four different DHAP-dependent aldolases.

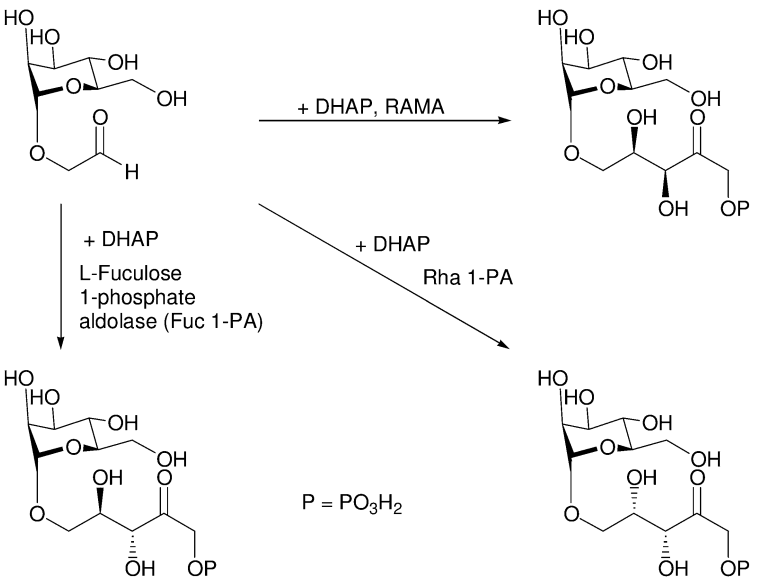
can be isolated from rabbit muscles is better known as RAMA (Rabbit Muscle Aldolase) [43].

FDP A was employed in a study of pancratistatin analogs to catalyze the formation of the D-threo stereochemistry (Scheme 5.24). When rhamnulose 1-phosphate aldolase (Rha 1-PA) was used the L-threo stereoisomer was obtained with excellent selectivity. Thus these two enzymes allow the stereoselective synthesis of the two threo-stereoisomers [44]. They were also utilised successfully for the synthesis of different diastereoisomers of sialyl Lewis X mimetics as selectin inhibitors. Not only the two threo-selective aldolases RAMA and Rha 1-PA, but also the D-erythro-selective Fuc 1-PA was employed. In this way it was possible to synthesise three of the four diastereoisomers enantioselectively (Scheme 5.25). The L-erythro stereochemistry as the only remaining diastereoisomer was not prepared [45]. This is because the aldolase that might catalyze its formation, TDP A, is not very stereoselective and therefore often yields mixtures of diastereoisomers.

A significant drawback of the DHAP-dependent aldolases is that DHAP cannot be replaced by dihydroxyacetone. DHAP is expensive and it is labile at neutral and basic pH values. It can be synthesized chemically, however the procedures are not very atom efficient. Therefore alternative enzyme-catalyzed approaches have been developed. A variety of them can be performed in the

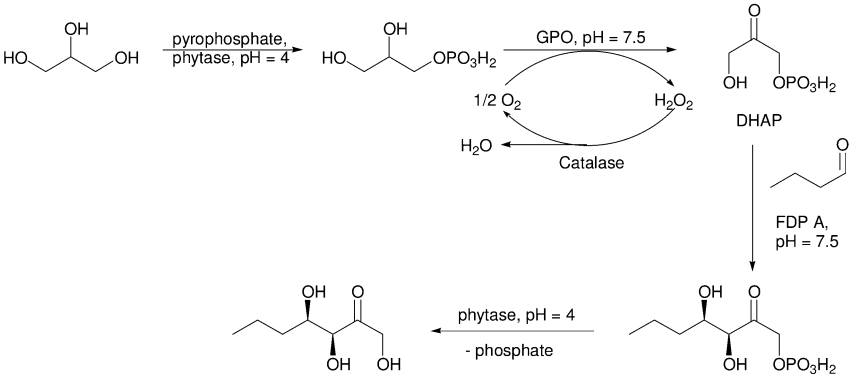


Scheme 5.24 Aldolase-catalyzed synthesis of different pancrastatin analogues.



Scheme 5.25 Aldolase-catalyzed synthesis of three stereoisomers of sialyl Lewis X mimetics.

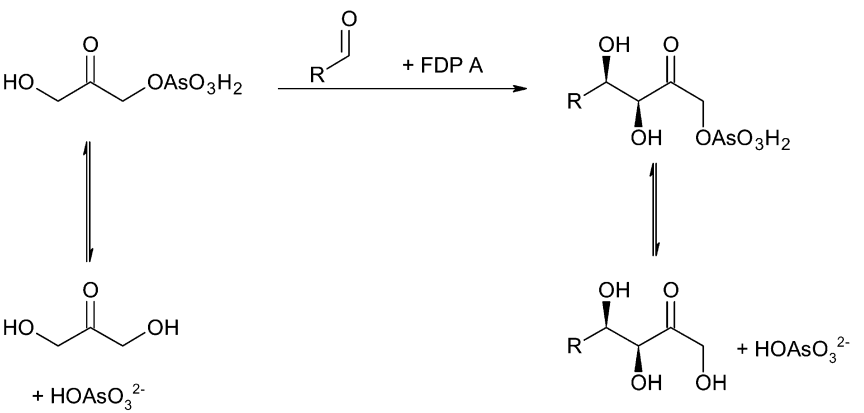
presence of the aldolase, allowing one-pot procedures. When starting from glycerol at lower pH values phytase can be employed for its selective phosphorylation, utilising cheap pyrophosphate as the phosphate source. Upon increase in the pH, glycerolphosphate oxidase (GPO) comes into action, catalyzing the formation of the desired DHAP and hydrogen peroxide. While FDP A catalyzes the enantioselective aldol reaction to yield the 5-deoxy-5-ethyl-D-xylulose monophosphate a catalase rapidly destroys the side product, thus preventing any oxidative damage. When the pH is lowered further, the phytase becomes active once more and catalyzes the dephosphorylation of the aldolproduct, thus the desired 5-deoxy-5-ethyl-D-xylulose is prepared in one pot from glycerol, pyrophosphate and butanal, catalyzed by four different enzymes [46]. Their efficient cooperation in one pot is controlled by the variation in pH levels (Scheme 5.26).



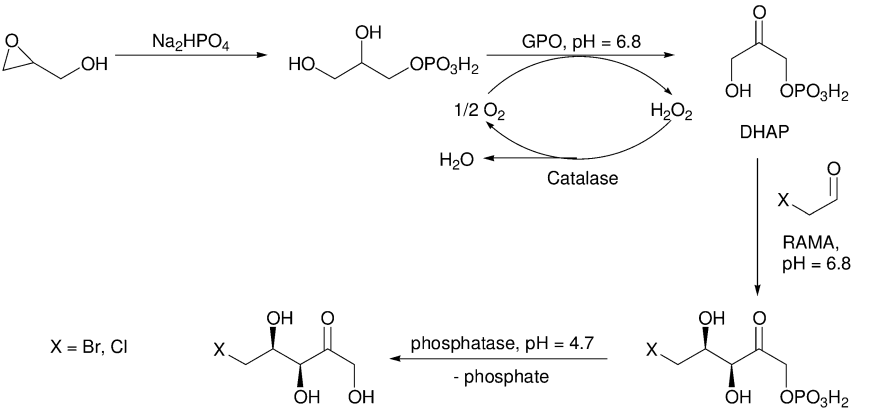
Scheme 5.26 The careful control of the pH value allows a one-pot four enzyme cascade; DHAP is prepared *in situ* and the product is deprotected by phytase.

The same authors then introduced a second system, in which arsenate esters were used as phosphate ester mimics. Dihydroxyacetone readily reacts with arsenate and the resulting ester is accepted by FDP A as a substrate. Since the arsenate ester formation is an equilibrium reaction the desired product is released *in situ* and the arsenate is available again for the next catalytic cycle (Scheme 5.27) [47]. However, although only catalytic amounts of arsenate are necessary it remains a toxic metal.

Recently a variation of the above described approach was introduced. *Rac*-glycidol was treated with disodiumhydrogenphosphate and then oxidized by catalase and L-glycerophosphate oxidase to DHAP. These two steps were integrated with a RAMA-catalyzed step, yielding the phosphorylated aldol product. After adjusting the pH, phosphatase was added and the aldol could be isolated in good yield (Scheme 5.28) [48]. It can be assumed that all the above-mentioned



Scheme 5.27 Arsenate esters can replace phosphate in DHAP.



Scheme 5.28 Four enzyme-catalyzed synthesis of aldol products.

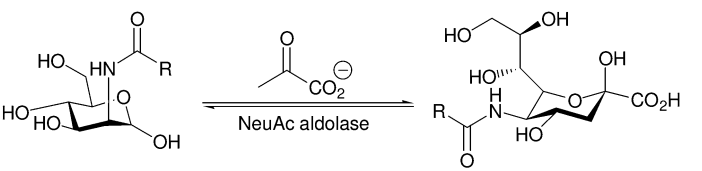
phosphorylation procedures can be coupled with other aldolases, giving access to the other stereoisomers.

Another problem of the DHAP-dependent aldolases is that the product is phosphorylated. The aldol reaction has therefore to be followed by a dephosphorylation step. In the sequences in Schemes 5.26–5.28 this deprotection is performed *in situ*. Otherwise several enzymes, such as phytase, are available for this purpose and the reaction normally proceeds under mild conditions [40, 43, 46].

5.2.4.2 **PEP- and Pyruvate-dependent Aldolases**

Pyruvate-dependent aldolases catalyze the breaking of a carbon–carbon bond in nature. This reaction can, however, be reversed if an excess of pyruvate is used, establishing one new stereocenter in the course of it. The natural function of phosphoenolpyruvate (PEP)-dependent aldolases on the other hand is to catalyze the synthesis of α-keto acids. Since PEP is a very reactive, unstable and difficult to prepare substrate, they are not commonly used in synthesis.

N-Acetylneuraminic acid aldolase (NeuAc aldolase) is commercially available and has been the subject of much attention [49]. NeuAc aldolase catalyzes the aldol reaction between pyruvate and mannose or mannose derivatives. The enzyme activates the donor as its enamine, similar to the Type I aldolase described above (Scheme 5.21). The enzyme has been used for the synthesis of aza sugars and var-



Scheme 5.29 Industrial synthesis of *N*-acetylneuraminic acid.

ious sialic acid derivatives and it is therefore also known as sialic acid aldolase. A number of chemo-enzymatic syntheses of sialic acid derivatives have been developed over the years. Acylated mannosamine derivatives could be converted into sialic acids with a variety of substituents at C-5 (Scheme 5.29). The versatility of this aldolase is best demonstrated by the fact that it is used for the multi-ton conversion of *N*-acetylmannosamine (R = Me) into *N*-acetylneuraminic acid [49–51].

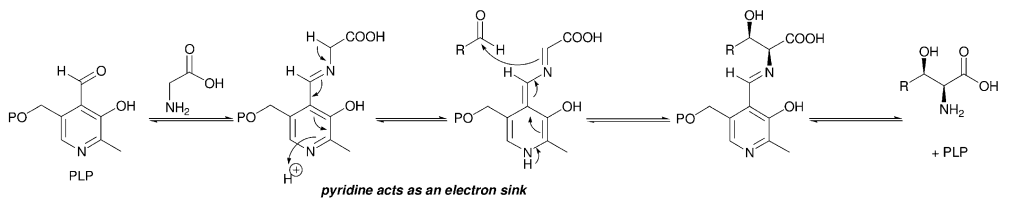
5.2.4.3 **Glycine-dependent Aldolases**

The glycine-dependent aldolases contain a cofactor: pyridoxal phosphate (PLP). Binding of glycine to it as an imine enables the deprotonation necessary for the carbon–carbon bond forming reaction, with pyridine acting as an electron sink. The subsequent 100% atom efficient reaction with an aldehyde establishes the new bond and two new stereocenters (Scheme 5.30). Of all the glycine-dependent aldolases only L-threonine aldolase (LTA) is commonly used [40, 43, 52].

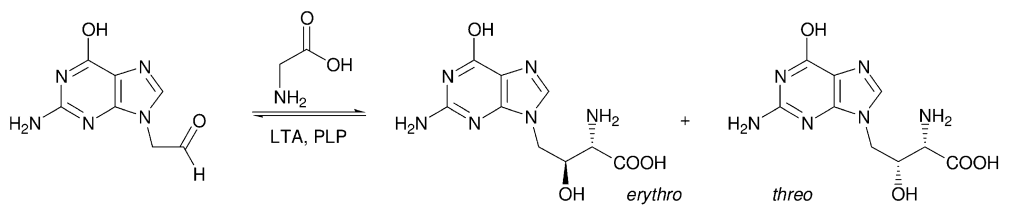
LTA has been successfully employed for the synthesis of peptide mimetics (Scheme 5.31). This example also reveals the drawback of this enzyme [53]. It only establishes the stereochemistry of the amino group, the second stereocenter, however, is not well defined. Thus diastereomeric mixtures of *threo* and *erythro* products are obtained, although the enzyme was evolved by nature to catalyze the formation/destruction of the *threo* product.

5.2.4.4 **Acetaldehyde-dependent Aldolases**

Acetaldehyde-dependent aldolases are the only aldolases that can catalyze the aldol formation between two aldehydes, i.e. with an aldehyde both as donor and acceptor [2–4, 40, 43]. More importantly, since they utilize, with high selectivity,



Scheme 5.30 Mechanism of the glycine-dependent aldolases.

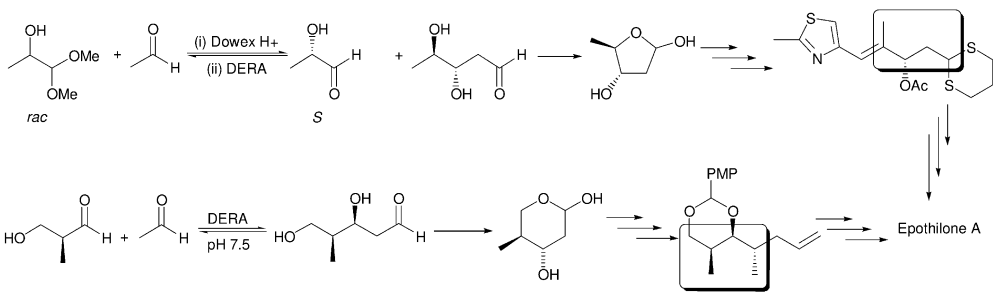


Scheme 5.31 Application of LTA in synthesis.

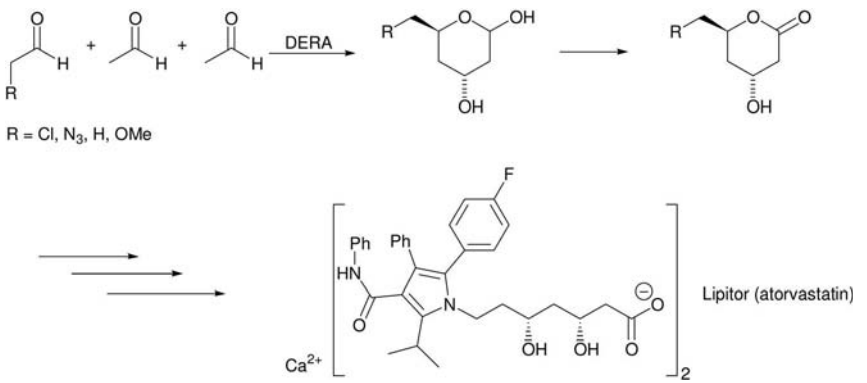
acetaldehyde as a donor, many cross coupling reactions are possible. To date only one of these aldolases has been applied extensively in organic synthesis: 2-deoxyribose-5-phosphate aldolase (DERA). DERA is a Type I aldolase: the active site lysine attacks acetaldehyde and activates the donor as an enamine (Scheme 5.21). It seems that a water molecule present in the active site plays an essential role in the catalysis and that the reaction mechanism is like that of DHAP-dependent Type I aldolases [54]. Also similar to the DHAP-dependent aldolases, DERA accepts a broad range of acceptors. The stereochemistry at the α-carbon of the acceptor as well as the polarity of the substituent determines whether the acceptor is a good substrate for DERA. If racemic aldehydes are used DERA often catalyzes the conversion of only one enantiomer, performing a kinetic resolution. However, if the "wrong" enantiomer of the acceptor is used as the sole substrate, DERA does occasionally convert it, too [55].

A particularly successful synthesis of Epothilone A is based on two DERA-catalyzed steps. In these two of the seven stereocentres of Epothilone A were established. When a racemic aldehyde was released *in situ* from its acetal, DERA converted only the *R*-enantiomer into the stable cyclic hemiacetal. This is a combined kinetic resolution and carbon–carbon bond formation yielding a building block with two chiral centers. Since the alcohol function was oxidized, the optical information obtained from the kinetic resolution was lost. Thus, for the overall yield it would have been better if DERA had displayed no stereoselectivity towards the acceptor (Scheme 5.32). In the DERA-catalyzed synthesis of another part of Epothilone A DERA is again highly stereoselective. Fortunately its preference is for the *S*-enantiomer of the acceptor aldehyde, the enantiomer that has to be submitted to the carbon–carbon bond formation in order to obtain the desired building block, again a stable hemiacetal (Scheme 5.32). Indeed, both DERA-catalyzed reactions yield open chain products that form stable cyclic hemiacetals. This ensures that the equilibria of these aldol reactions are shifted towards the desired products. Further synthetic manipulations converted these intermediates into Epothilone A [55].

A particularly elegant application of DERA is the sequential synthesis of thermodynamically stable cyclic hemiacetal. Two DERA-catalyzed aldol reactions convert one equivalent of acceptor and two equivalents of acetaldehyde into this stable compound. A mild subsequent oxidation yielded the corresponding lactone in ex-



Scheme 5.32 DERA catalyzes key steps in the synthesis of Epothilone A.

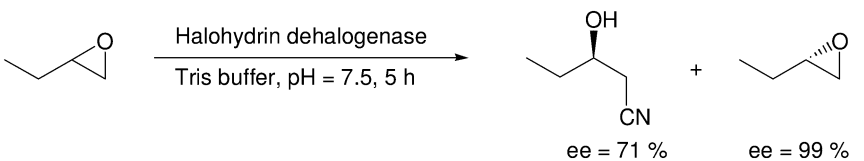


Scheme 5.33 DERA-catalyzed enantioselecitive synthesis of the atorvastatin side chain.

cellent optical purity, proving the great versatility of this class of enzymes. The lactones with $R = N_3$ or Cl are intermediates for the synthesis of Lipitor (atorvastatin), a cholesterol-lowering drug (Scheme 5.33) [56]. A mutant DERA has been employed in this synthesis and alternatively the screening of a very diverse library of environmental samples was performed and the DERA obtained from this library was used. The DERA derived from the screening was utilized for further optimisation and a process was developed in which the aldolase-catalyzed reaction cascade proceeds with an enantioselectivity of over 99% and the *de* of the product is above 96%. Equally important, the reaction proceeds at high substrate concentrations and up to 30 g L^{-1} h^{-1} of product are obtained [57]. Currently this is one of the most competitive approaches to the chiral side chain of the statins [58, 59].

5.2.5
Enzymatic Synthesis of *β*-Hydroxynitriles

Recently it was described that the halohydrin dehalogenases can be employed for the enantioselective formation of a new carbon–carbon bond. Their natural task is the dehalogenation of halohydrins, however this reaction can be reversed. When utilizing cyanide instead of a halide as the nucleophile in the reverse reaction the desired *β*-hydroxynitrile is formed with good to excellent enantioselectivity (Scheme 5.34) [60].



Scheme 5.34 Application of a halohydrin dehalogenase for the synthesis of a carbon–carbon bond.

5.3
Transition Metal Catalysis

While the application of enzymes and proline as catalysts for the (commercial) formation of carbon–carbon bonds is relatively new, transition metal catalysts are well established for the industrial synthesis of carbon–carbon bonds. Although in themselves not always perfectly green, transition metal catalysts often allow the replacement of multi-step and stoichiometric reaction sequences with one single catalytic step. Thus, the overall amount of waste generated and energy used is reduced drastically [61–64].

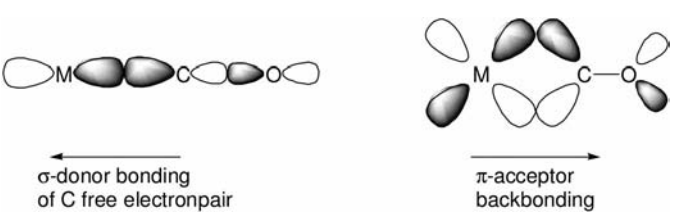
5.3.1
Carbon Monoxide as a Building Block

Carbon monoxide is a readily available C-1 building block that can be generated from many different sources [61]. It is, however, also a highly toxic gas and does not therefore fulfill the criteria of green chemistry as given in the working definition in Chapter 1, Section 1.1. Similar to HCN, it fortunately can be safely handled on large and particularly industrial scales, making it a very attractive reagent for synthesis. Recently strategies for the replacement of carbon monoxide have been developed. In particular methyl formate can be used as a substitute and the current state of the art has been reviewed [65].

Carbon monoxide is an excellent ligand for transition metals. It coordinates via the free electron pair of the carbon atom as a σ-donor (and weakly as a π-donor) to the metal, back bonding shifts electrons from the transition metal into the π^* antibonding orbital of carbon monoxide, making it susceptible to the catalytic chemistry discussed here (Scheme 5.35).

5.3.1.1 **Carbonylation of R–X (CO "Insertion/R-migration")**

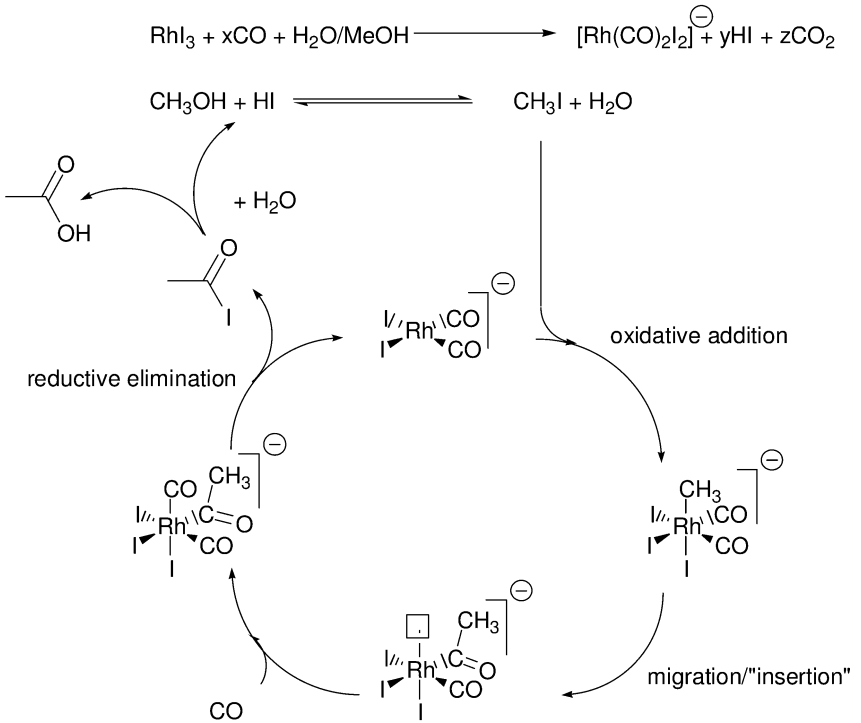
A long-standing success in transition metal catalysis is the carbonylation reaction [66], in particular the synthesis of acetic acid [67]. Formally this is the insertion of CO into another bond, in particular into a carbon–halogen bond. After the oxidative addition to the transition metal (the breaking of the carbon–halogen bond), a reaction with a CO ligand takes place. This reaction is often called an insertion. Mechanistic studies have, however, shown that the actual reaction



Scheme 5.35 Bonding and backbonding in metal carbonyl complexes.

is a migration of the alkyl or aryl group to the CO ligand, not an insertion of the CO in the transition metal carbon bond [61, 63]. This generates a vacant site on the transition metal catalyst, which will be taken up by a new ligand. A subsequent reductive elimination releases the "insertion product" (Scheme 5.36).

Mankind has produced acetic acid for many thousand years but the traditional and green fermentation methods cannot provide the large amounts of acetic acid that are required by today's society. As early as 1960 a 100% atom efficient cobalt-catalyzed industrial synthesis of acetic acid was introduced by BASF, shortly afterwards followed by the Monsanto rhodium-catalyzed low-pressure acetic acid process (Scheme 5.36); the name explains one of the advantages of the rhodium-catalyzed process over the cobalt-catalyzed one [61, 67]. These processes are rather similar and consist of two catalytic cycles. An activation of methanol as methyl iodide, which is catalytic, since the HI is recaptured by hydrolysis of acetyl iodide to the final product after its release from the transition metal catalyst, starts the process. The transition metal catalyst reacts with methyl iodide in an oxidative addition, then catalyzes the carbonylation via a migration of the methyl group, the "insertion reaction". Subsequent reductive elimination releases the acetyl iodide. While both processes are, on paper, 100%



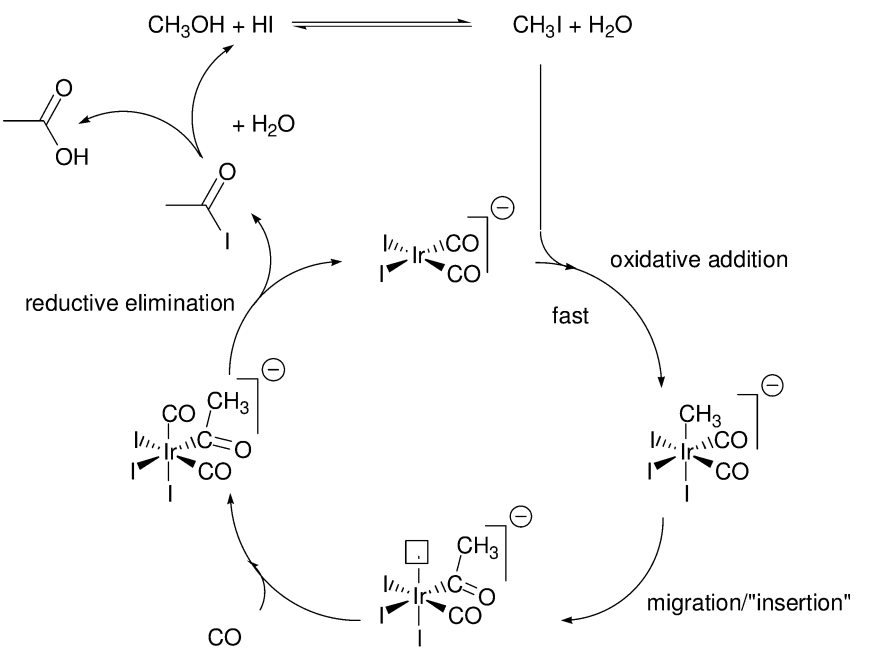
Scheme 5.36 The Monsanto acetic acid process.

atom efficient, methanol and CO are not used in a ratio 1:1 and there was room for improvement in particular for the CO conversion [68].

With the introduction of an iridium catalyst the carbonylation of methanol could be further improved [68, 69]. While the basic process remains the same, i.e. methanol is first converted into methyl iodide, the iridium-catalyzed carbonylation (Cativa process, Scheme 5.37) differs in several ways from the other processes. The selectivity of the reaction when correlated to methanol is greater than 99% but even for CO the yields are above 90%, indicating that the Cativa process is not only theoretically highly atom efficient. This improved selectivity improves the product purity and reduces the work-up costs. In addition, the water concentration in the reactor could be reduced, again easing the work up. Moreover, the iridium catalyst is more active that either rhodium or cobalt-catalyst and significantly more stable, ensuring a longer lifetime [69].

Next to the large-scale application for bulk chemical production, carbonylation reactions have successfully been applied for the synthesis of fine chemicals. In particular the synthesis of Ibuprofen, an analgesic and anti-rheumatic drug could be greatly improved. Instead of the wasteful multi-step Darzen's glycidic ester condensation reaction that was originally employed for the introduction of the acid group a formal insertion of carbon monoxide into the benzylic alcohol drastically shortened the synthesis [70–72].

The reaction proceeds in MEK and conc. HCl at 130 °C. First, the alcohol is converted into the benzylic chloride. In this case a palladium catalyst

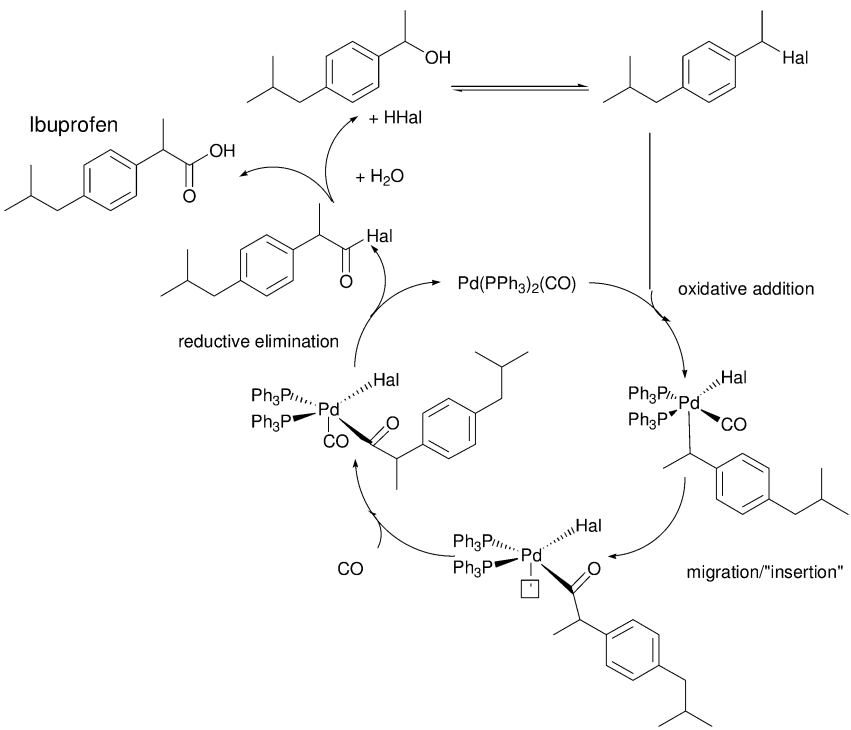


Scheme 5.37 The CATIVA process.

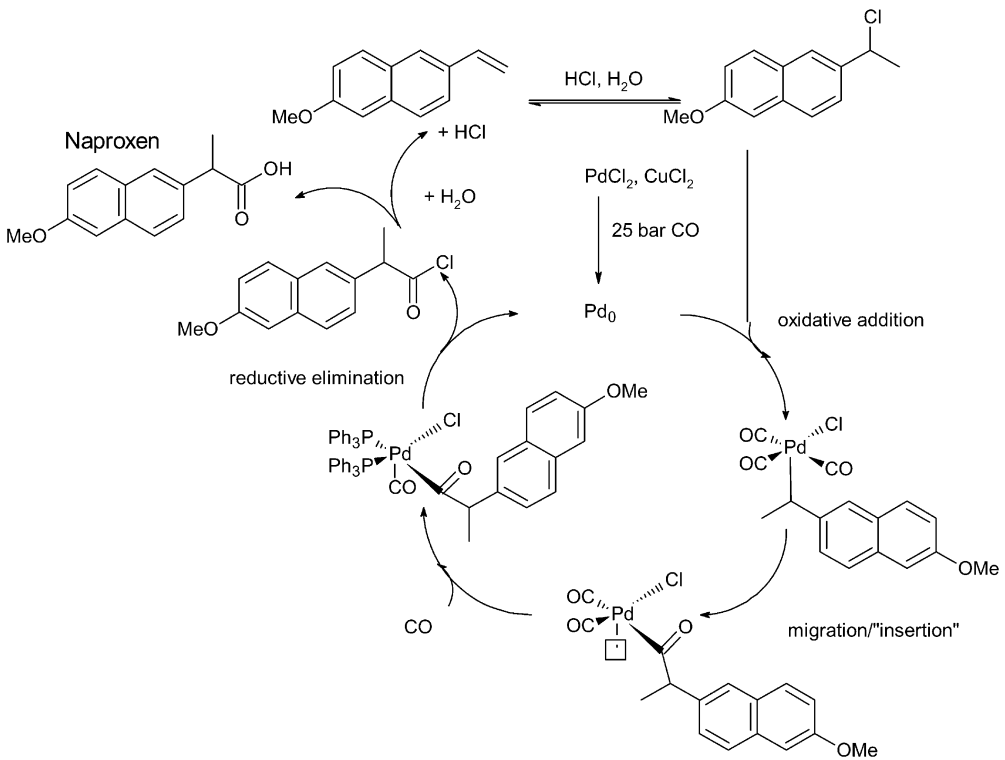
$PdCl_2(PPh_3)_2$ was applied. After initial reduction of the palladium to obtain the active species, an oxidative addition of the benzylic chloride takes place. Migration of the benzylic group leads to the formation of the new carbon–carbon bond; a reductive elimination then releases ibuprofen as its acid chloride which is hydrolysed, closing the second catalytic cycle, too. This process is used to produce 3500 t/year of ibuprofen and TON of 10 000 have been reached for the palladium catalyst (Scheme 5.38).

In a similar approach, naproxen is prepared from an olefin, the product of a Heck reaction (see Section 5.3.2.1). As described above, the reaction proceeds in the presence of water and HCl, additionally copper(II)chloride is added, possibly to prevent the formation of palladium black (Scheme 5.39). Addition of HCl to the double bond and subsequent oxidative addition reaction of the benzylic chloride with the active Pd^0 species initiates the catalytic cycle, which proceeds similarly to the ibuprofen synthesis [70–73].

In an attempt to replace the organohalides as starting materials, diazonium salts have been investigated as starting material. They, too, can react in an oxidative addition with the Pd^0 to form the first intermediate of the catalytic cycle. In a recent bench scale process for a herbicide in development (CGA 308 956) a diazonium salt proved to be suitable. The product of the oxidative addition will



Scheme 5.38 Industrial carbonylation in the Ibuprofen synthesis of Celanese.

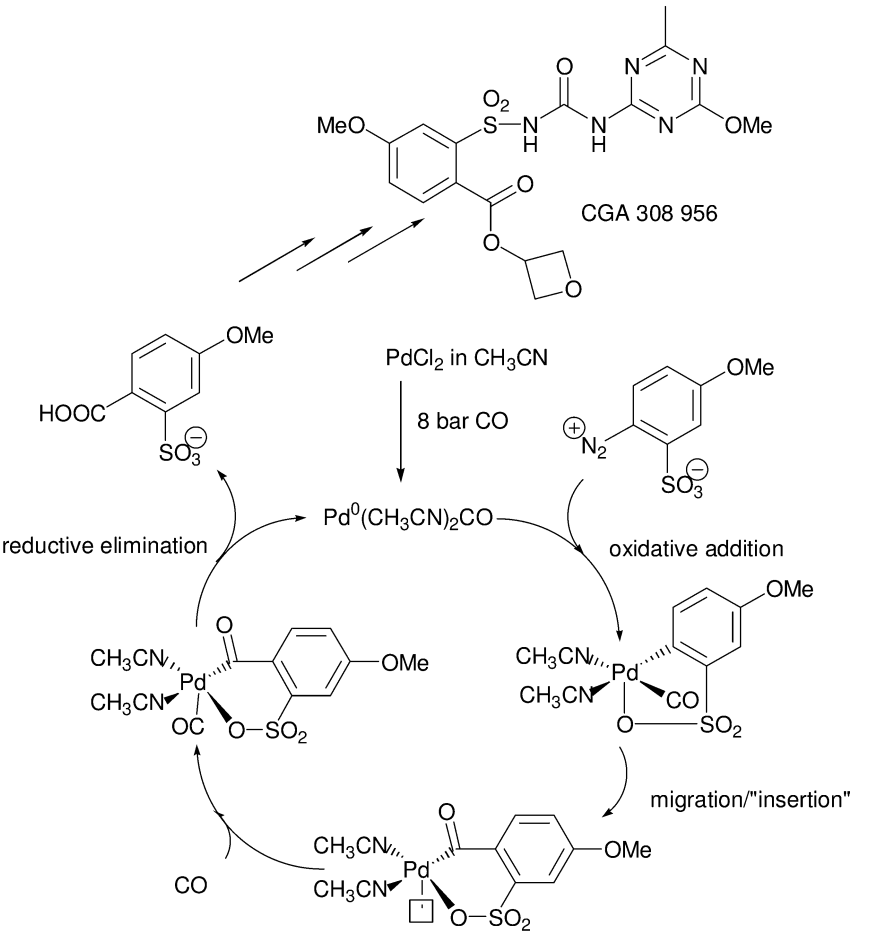


Scheme 5.39 Naproxen synthesis by Albemarle.

most likely be a complex with a bidentate ligand, the sulfonic acid group being in close proximity to the Pd. This oxidative addition also releases nitrogen, which causes an increase in pressure during the reaction, however this can readily be controlled Scheme 5.40). 2-Sulfo-4-methoxybenzoic acid (SMBA) was synthesized in excellent yields with 1 mol% palladium chloride (TON 100, TOF 30 h^{-1}), acetonitrile was employed as solvent and ligand for the catalyst [70, 74].

5.3.1.2 **Aminocarbonylation**

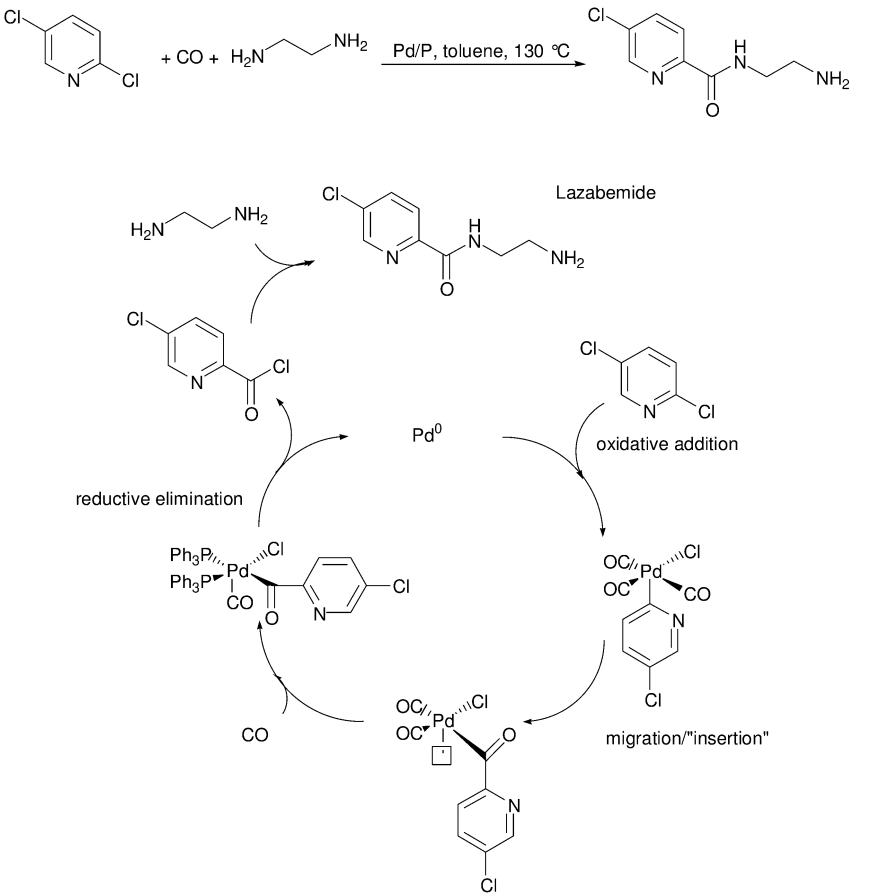
In the above described carbonylation reactions the nucleophile that reacts with the species released from the catalytic cycle is water or possibly an alcohol. This can be replaced by a more nucleophilic amine, yielding an amide as the product [66]. With this minor variation a different group of products becomes accessible. A striking application of this reaction is the synthesis of the monoamine oxidase B inhibitor Lazabemide [70, 75]. The first laboratory synthesis could be shortened from 8 steps to just one catalytic reaction with a TON of 3000 (Scheme 5.41). The only drawback to the greenness of this reaction is that the metal is removed via an extraction with aqueous NaCN.



Scheme 5.40 Organohalides can be replaced by diazonium salts.

5.3.1.3 **Hydroformylation or "Oxo" Reaction**

Hydroformylation, also known by its old name as "oxo" reaction, is the carbonylation of olefins in the presence of hydrogen, i.e. treatment of olefins with synthesis gas (syn gas). Formally it is the addition of a formyl group and one hydrogen atom to a carbon–carbon double bond [61, 76, 77]. This 100% atom efficient reaction was described as early as 1938. At 100–400 bar and 90–250 °C, olefins, in particular ethylene (Scheme 5.42), were converted into the corresponding aldehydes. $HCo(CO)_4$ generated from $Co_2(CO)_8$ was used as catalyst, but these reactions had several disadvantages, among them a relatively low selectivity for the n-aldehyde rather than the isoaldehyde, the volatility of the cobalt catalyst and loss of alkene due to hydrogenation. Nowadays this carbon–carbon bond formation is normally performed with a rhodium catalyst. These tend to be 10^4 times faster than the comparable cobalt catalysts. A detailed description of a particularly green hydrofor-

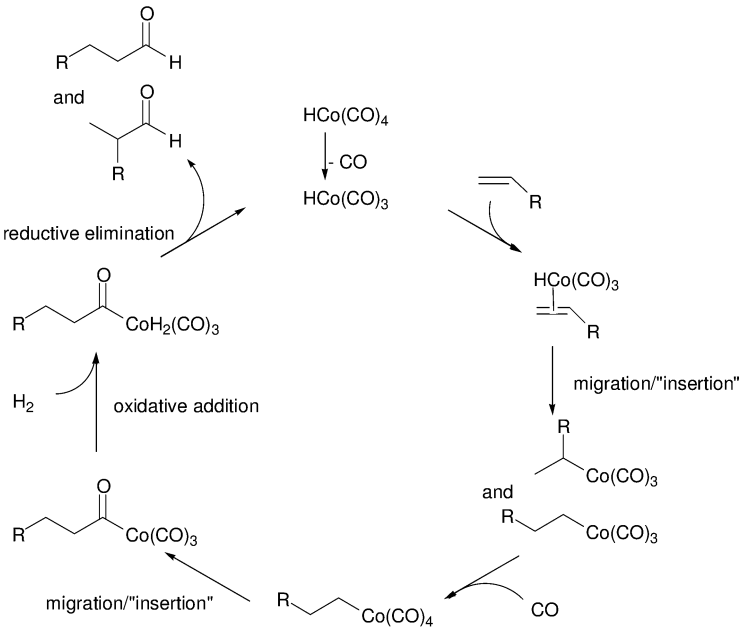


Scheme 5.41 Hoffmann-La Roche production of Lazabemide

mylation, the Rh-catalyzed Ruhrchemie/Rhone-Poulenc process (performed in water), is given in Chapter 7, Section 7.3.1.

5.3.1.4 **Hydroaminomethylation**

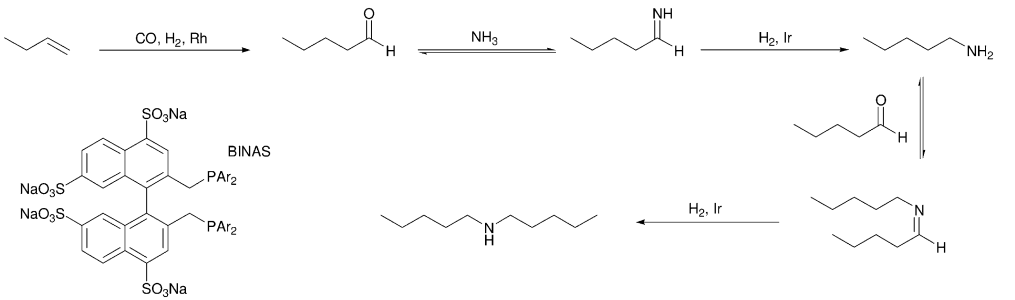
An interesting variation of hydroformylation with a great potential for the industrial preparation of primary amines is hydroaminomethylation. In this process two catalytic reactions are combined, a hydroformylation and a reductive amination of the resulting aldehyde. Although first described more than 60 years ago a really successful procedure was only published recently [78]. To ensure the success of this sequence a rhodium catalyst for the hydroformylation was combined with an iridium catalyst for the imine reduction in a two-phase system, similar to the Ruhrchemie/Rhone-Poulenc process for the hydroformylation. It was demonstrated that less polar solvents such as toluene in combina-



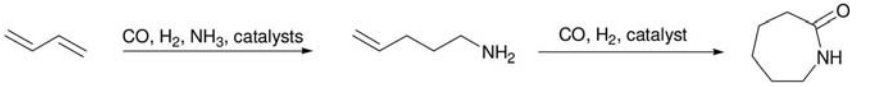
Scheme 5.42 Cobalt-catalyzed hydroformylation.

tion with water and high ammonia concentrations lead to very good selectivities for primary amines. When an ammonia/alkene ratio of 0.5 was employed in combination with BINAS as water-soluble ligand not only were almost pure secondary amines obtained but they had n:iso ratios of 99:1. Overall this approach is opening new opportunities for the green industrial preparation of low molecular mass primary or secondary amines (Scheme 5.43).

Indeed hydroaminomethylation is part of a new route to ε-caprolactam. Starting with readily available butadiene it enables the first carbon–carbon bond formation, extending the carbon chain and introducing the aminogroup. In a sub-



Scheme 5.43 A two-phase approach enabled the first efficient hydroaminomethylation.



Scheme 5.44 A new route to nylon?

sequent cobalt-catalyzed intramolecular aminocarbonylation the lactam is formed. It was demonstrated that the addition of trialkylphosphines improved the formation of the lactam while suppressing the formation of the polymer [79]. This process is, however, still in the development stage (Scheme 5.44).

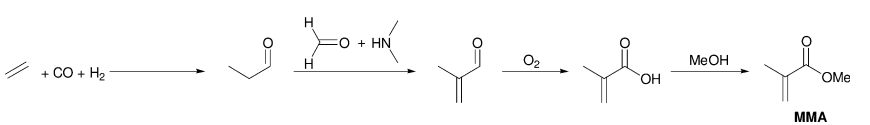
5.3.1.5 **Methyl Methacrylate via Carbonylation Reactions**

Methyl methacrylate (MMA) is one of the most important monomers [80–82]. It forms the basis of acrylic plastics and of polymer dispersion paints. The traditional production is by the formation of acetone cyanohydrin, elimination of water and hydrolysis of the nitrile group, followed by the ester formation. In the carbon–carbon bond forming reaction large amounts of excess HCN and ammonium bisulfate are left as waste. Although these problems have been addressed there is still much room for improvement. In particular the number of reaction steps should be reduced and, in order to achieve this, cyanide should be avoided. The building block to replace it is CO.

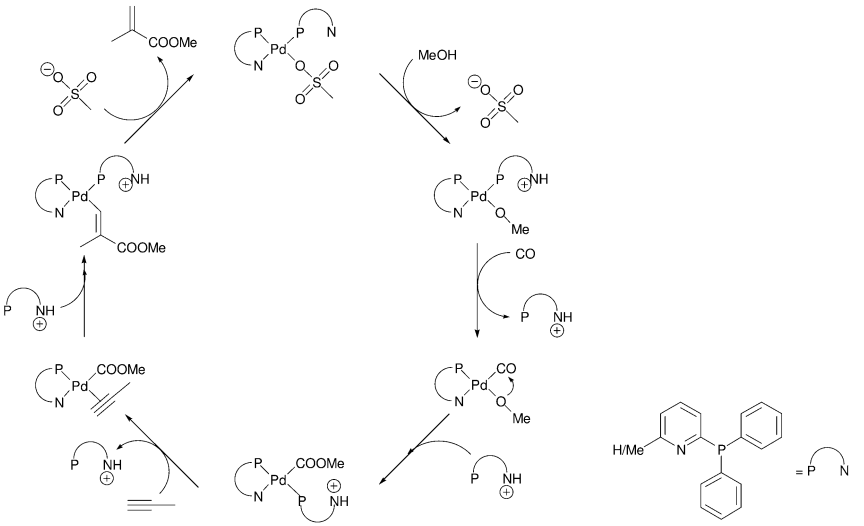
Indeed, this has been realised and BASF started a plant on a 5000 ton/year scale in 1989 [81, 82]. The process is based on the hydroformylation of ethylene. Subsequently the propanal is converted via a Mannich reaction into methacrolein, oxidation and esterification then leads to MMA (Scheme 5.45).

A much shorter route is the Reppe carbonylation [83] of propyne. Propyne is, together with propadiene (allene), part of the C3 stream of the cracking process. The order of metal substrate binding strength is allenes > alkynes > alkenes. Thus the desired reaction can only proceed if the propadiene has been removed from the feed, since it is an inhibitor of the Pd catalyst. Equally important, the alkyne complex reacts much faster than the alkene complex. Thus the product is neither a substrate nor an inhibitor for the catalyst (Scheme 5.46).

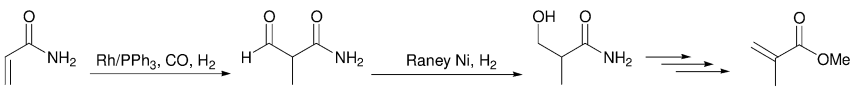
Pd complexed with 2-pyridyldiphenylphosphine (or its 6-methylpyridyl derivative) and weakly coordinating counter ions, such as methylsulfonate, are employed as catalyst [84, 85]. After the coordination of methanol and CO the migration of the methoxy group takes place to form the palladium carbomethoxy species. Then the coordination of propyne takes place. The steric bulk of the pyridyl group, and in particular of the 6-methylpyridyl group, induces the con-



Scheme 5.45 BASF route to MMA.



Scheme 5.46 Reppe carbonylation leads directly to MMA.



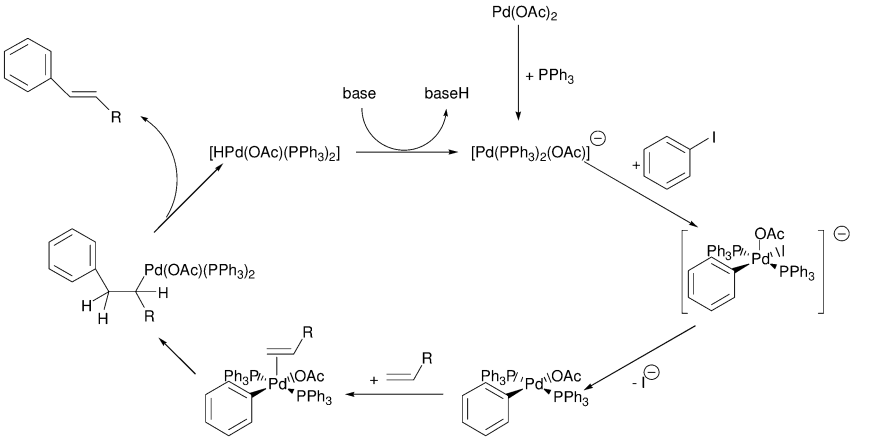
Scheme 5.47 MMA from acrylamide.

figuration necessary to achieve the regiochemistry in the migratory insertion reaction. Thus the carbomethoxy group adds to the triple bond forming 99.95% pure MMA and only traces of methyl crotonate. Although this process has been heralded as a green way to MMA it still awaits industrial application.

Recently a new approach towards MMA was proposed. Starting from acrylamide, a Rh-catalyzed hydroformylation yielded, with high selectivity, the desired branched aldehyde. Given the many possible side reaction this is an achievement in its own right. Addition of Raney Ni allowed a sequential reduction of the aldehyde [86]. The alcohol obtained in this manner can then be converted via standard reactions into MMA (Scheme 5.47). Whether this approach does not involve, once again, too many steps remains to be seen.

5.3.2
Heck-type Reactions

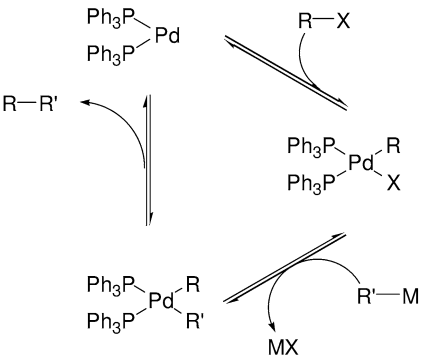
The coupling of aromatic rings with side chains is traditionally performed with Friedel-Crafts reactions (Chapter 2). With the introduction of the Heck reaction it is now possible to perform this type of carbon–carbon bond formation under mild conditions, in the presence of many functional groups and without the waste that is associated with the use of Lewis acids such as $AlCl_3$. Similarly, Pd-



Scheme 5.48 Catalytic cycle of the Heck reaction.

catalyzed carbon–carbon bond forming reactions such as the Suzuki and the Sonogashira coupling can replace the Ullman, Grignard and Glaser coupling.

The Heck reaction is catalyzed by Pd^0 and a base [72, 73, 87–93]. An oxidative addition of an arylhalide to Pd^0 constitutes the first step, followed by the coordination of the olefin. The migratory insertion forms the new carbon–carbon bond and a β-elimination releases the desired product. Subsequent deprotonation reduces the Pd^{2+} to Pd^0 again, regenerating the catalyst (Scheme 5.48). In the Suzuki [94] and Sonogashira, as well as in the Negishi and Stille coupling the first step is identical [62]. An organohalide reacts in an oxidative addition with Pd^0. It is essential that the organohalide has no β-hydrogen atoms since this would allow a rapid β-elimination, preventing the desired coupling reaction. Instead of employing an olefin, like in the Heck reaction, an organometallic compound is used. In the Suzuki reaction this is an organoboronic acid, in the Sonogashira reaction a Cu^+ organyl is employed, while tin organyls and zinc or-



Scheme 5.49 Catalytic cycle of the Suzuki, Stille, Negishi and Sonogashira reactions.

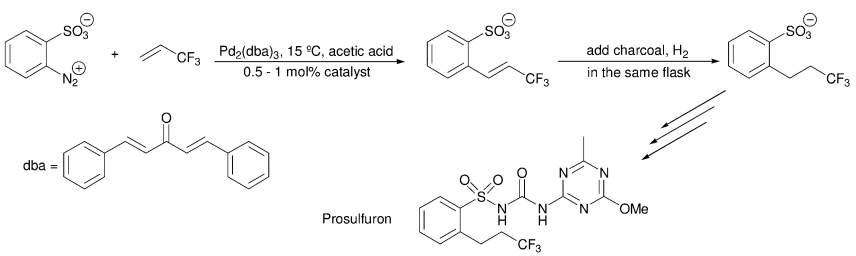
ganyls are used in the Stille and the Negishi reactions, respectively. Transmetallation then leads to a Pd species carrying the two organic groups that are coupled in a reductive elimination (Scheme 5.49). All four coupling reactions have proven their value in organic synthesis, but given the high toxicity of tin the Stille reaction will not be discussed here. Due to the limited industrial application of the Negishi reaction it will also not be included in further discussion.

5.3.2.1 **Heck Reaction**

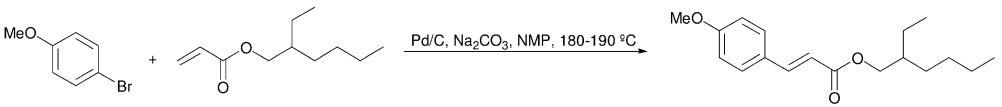
The great advantage of the Heck reaction is that it enables syntheses of fine chemicals that would otherwise only be accessible via long and wasteful routes. The first reported industrial Heck reaction is the Matsuda-Heck coupling of a diazonium salt, part of the Ciba-Geigy synthesis of the herbicide Prosulfuron [70, 73]. The application of a diazonium salt instead of an arylhalide reduces the amount of salts generated, allowing a particularly green reaction. Subsequent to the carbon–carbon bond formation, charcoal is added to remove the Pd. This at the same time generates the catalyst for the next step, a catalytic hydrogenation. Thus the expensive Pd is used twice and can then be recycled, significantly reducing costs (Scheme 5.50). Indeed this was and is one of the main problems in Pd-catalyzed reactions, to find catalysts that are so efficient that the application of this expensive metal is viable on an industrial scale.

A particularly straightforward way to circumvent the problems with catalyst recycling or the formation of palladium black from the homogeneous Pd catalysts is to use Pd on charcoal. This has been pioneered by IMI/Bromine Co Ltd for the industrial production of the UV-B sunscreen 2-ethylhexyl-*p*-methoxy-cinnamate (Scheme 5.51). At high temperatures *p*-bromoanisole is treated with 2-ethylhexylacrylate to yield the desired product and some side products – most likely due to the 180–190 °C reaction temperature. It is not quite clear what the actual catalytic species is [73, 95]. It can be assumed that separate Pd atoms that are released from the carrier at the elevated temperature are the catalytically active species.

Indeed, the observation that Pd black is often formed during Heck reactions led to detailed studies of the active species in many Heck reactions [96]. It was demonstrated that colloidal Pd, Pd that had not yet formed Pd black clusters



Scheme 5.50 First industrial application of the Heck reaction.

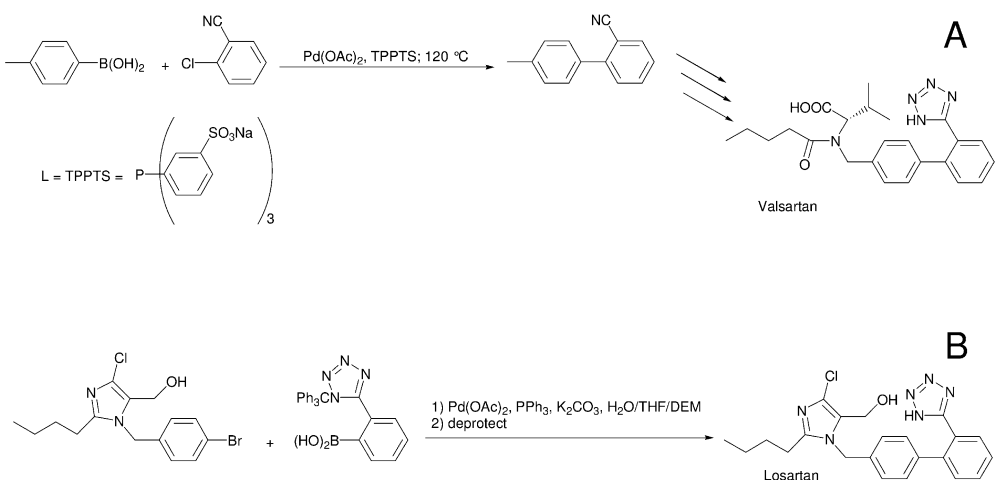


Scheme 5.51 Sunscreen production with immobilized Pd.

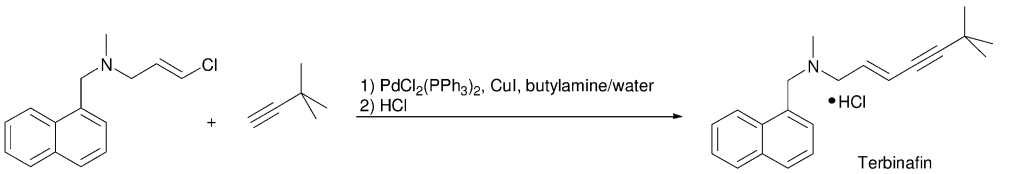
but was still in solution, did actually catalyze the reaction. This paved the way for the introduction of "homeopathic concentrations" of ligand-free Pd as catalyst of the Heck reaction [97, 98]. Very recently it was demonstrated that it is indeed the leached atoms from the Pd-clusters that catalyze the Heck reaction [99]. With this result in hand new variations of the Heck reaction are possible.

5.3.2.2 **Suzuki and Sonogashira Reaction**

The Suzuki coupling enables the high yield and clean linkage of two differently substituted aromatic rings, thus opening new opportunities for synthesis [94]. Based on this reaction many new entities could be synthesized and the Hoechst (now Clariant) process for 2-cyano-4′-methylbiphenyl [70, 72] forms the basis of the production of Valsartan and similar angiotensin II receptor antagonists (Scheme 5.52a). The reaction is catalyzed by a straightforward catalyst, Pd/sulfonated triphenylphosphine in water/DMSO/glycol at 120 °C. At the end of the reaction two phases form, the polar catalyst can thus be easily recycled. In the Merck synthesis of Losartan the Suzuki coupling is performed at a much later stage [100], linking two highly functionalized benzene rings with excellent selectivity (Scheme 5.52b), demonstrating the great flexibility of this carbon–carbon bond forming reaction.



Scheme 5.52 Industrial application of the Suzuki reaction.

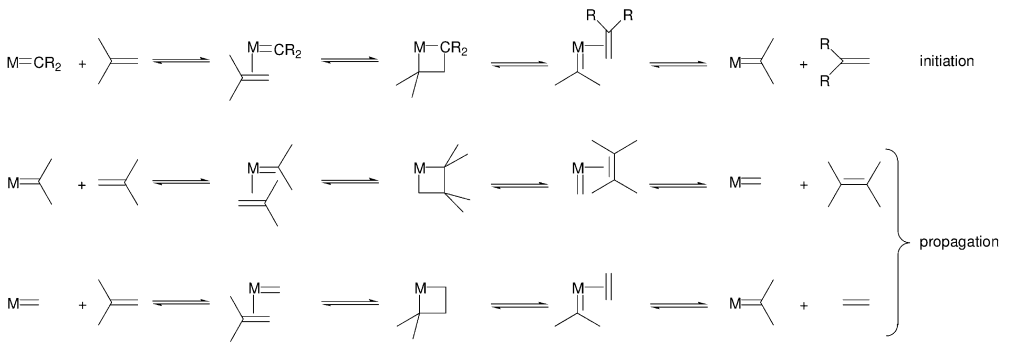


Scheme 5.53 Synthesis of the antifungal agent Terbinafin via Sonogashira coupling.

The Sonogashira coupling has enabled the efficient synthesis of Terbinafin, an antifungal drug [70, 72, 101]. The Sandoz/Novartis process is performed on a multi-ton scale, directly forming the drug. Less than 0.05 mol% of precatalyst $PdCl_2(PPh_3)_2$ in butylamine/water are used and $Cu^{I}I$ is the co-catalyst to activate the acetylene (Scheme 5.53).

5.3.3
Metathesis

More than half a century ago it was observed that Re_2O_7 and Mo or W carbonyls immobilized on alumina or silica could catalyze the metathesis of propylene into ethylene and 2-butene, an equilibrium reaction. The reaction can be driven either way and it is 100% atom efficient. The introduction of metathesis-based industrial processes was considerably faster than the elucidation of the mechanistic fundamentals [103, 104]. Indeed the first process, the Phillips triolefin process (Scheme 5.55) that was used to convert excess propylene into ethylene and 2-butene, was shut down in 1972, one year after Chauvin proposed the mechanism (Scheme 5.54) that earned him the Nobel prize [105]. Starting with a metal carbene species as active catalyst a metallocyclobutane has to be formed. The Fischer-type metal carbenes known at the time did not catalyze the metathesis reaction but further evidence supporting the Chauvin mechanism was published. Once the Schrock-type metal carbenes became known this changed. In 1980 Schrock and coworkers reported tungsten carbene complexes



Scheme 5.54 The Chauvin mechanism.

that catalyzed the metathesis reaction [106–108]. The introduction of the first generation Grubbs catalyst then made the metathesis reaction attractive to synthetic organic chemists [109, 110] and since then the metathesis reaction is used in bulk chemicals production and polymerization and also in fine-chemical synthesis, thus spanning the entire range of chemistry.

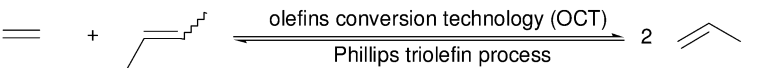
5.3.3.1 **Metathesis Involving Propylene**

As mentioned above, the first metathesis reaction studied was the equilibrium between propylene and an ethylene 2-butene mixture. In the initial Phillips process this was used to convert excess propylene into ethylene and 2-butene (Scheme 5.55). When propylene demands surged, the process was reversed and is now known as olefins conversion technology (OCT). The OCT process is operated with a fixed-bed reactor, WO_3 on silica serves as a catalyst. In order to allow 1-butene in the feed MgO is added as an isomerisation catalyst [104]. The process is a gas phase reaction, proceeding at >260 °C and 30–35 bar and is used globally on a several million ton per year scale. An alternative process has been developed by the Institut Français du Pétrole and the Chinese Petroleum Corporation of Taiwan. In a liquid phase reactor at 35 °C and 60 bar, ethylene and 2-butene are converted in the presence of Re_2O_7 and alumina.

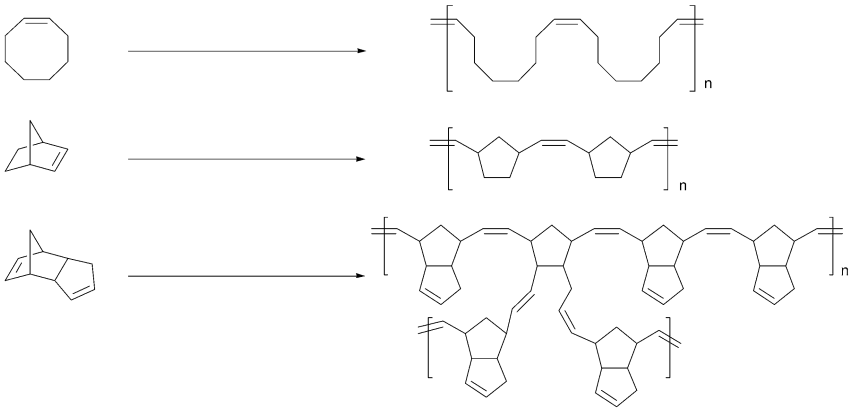
5.3.3.2 **Ring-opening Metathesis Polymerization (ROMP)**

When cyclic alkenes are utilized as starting materials the metathesis reaction will lead to long chain polymers and/or cyclic oligomers [103, 104, 107, 108]. If a strained cyclic alkene is employed the reaction is effectively irreversible. Industrially cyclooctene (polymer: Vestenamer), 2-norbornene (polymer: Norsorex), and dicyclopentadiene (polymer: Telene, Metton, Pentam) are used as monomers. Upon polymerization cyclooctene and 2-norbornene yield straight chain polymers while dicyclopentadiene also allows cross-linking (Scheme 5.56).

During the last years ROMP has been developed to generate self-healing polymers. In these polymers droplets of dicyclopentadiene and of Grubbs-catalyst are incorporated. When the polymer cracks the droplets burst open, the catalyst comes into contact with the monomer and the plastic ideally heals itself [111]. This methodology is still far from application but it does indicate the power of ROMP.



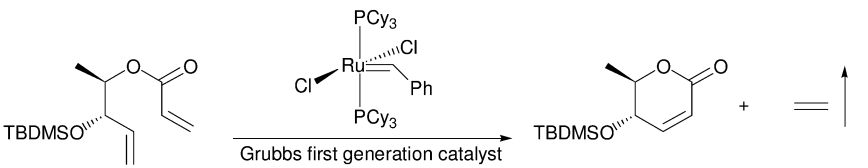
Scheme 5.55 First industrial application of the metathesis reaction.



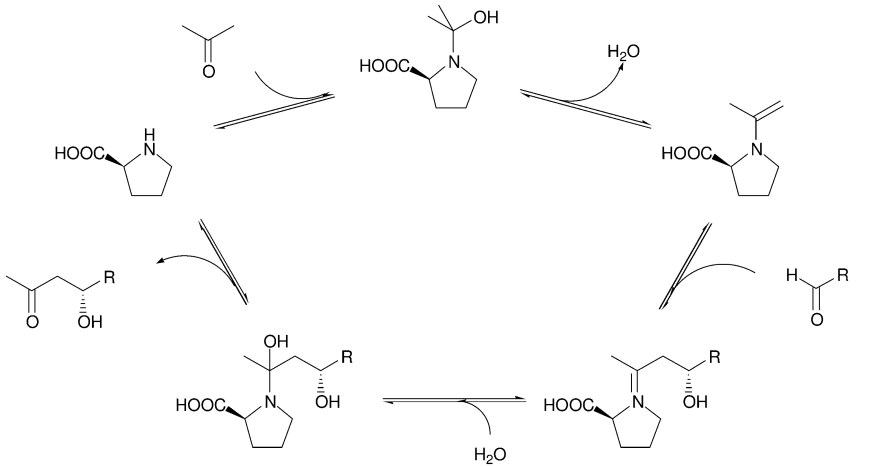
Scheme 5.56 ROMP as an approach to polymers.

5.3.3.3 **Ring-closing Metathesis (RCM)**

The metathesis reaction was introduced as a method to interconvert small olefins, such as ethylene, propylene and 2-butene. With a growing understanding of the mechanism and the introduction of defined catalysts, ROMP became established. Based on all the knowledge acquired, the opposite reaction could be developed, the ring-closing metathesis (RCM). One of the great challenges in natural product synthesis is the closure of large rings, often lactones or lactams. The classical approaches are often esterifications after the formation of amides, all this occurring in the presence of many delicate functional groups [108–110, 112]. RCM is a novel approach: an intramolecular metathesis reaction closes the ring, while releasing ethane, thus the reaction is virtually irreversible (Scheme 5.57). Given the highly improved metathesis catalysts, such as Grubbs first and second-generation catalyst, the reaction can now be applied in the presence of countless other functional groups. Thus an entirely new synthetic approach was introduced, opening up many new opportunities and establishing a new retrosynthetic disconnection.



Scheme 5.57 Ring-closing metathesis in the presence of functional groups.



Scheme 5.58 Proline-catalyzed aldol reaction.

5.4 Conclusion and Outlook

The formation of carbon–carbon bonds is at the heart of organic synthesis and essential to the production of bulk and fine chemicals as well as pharmaceuticals. During the first half of the twentieth century, transition metal catalyzed reactions were introduced for the production of small molecules and the first enantioselective enzymatic processes were established. More recently a new type of catalysis has entered the stage, organocatalysis. Central to its success is the extreme versatility of the amino acid proline, which almost seems to be able to enantioselectively catalyze every reaction of the aldehyde/keto group, and with high selectivity too. This has introduced a new approach to the aldol chemistry and many reports on this and similar reactions have been published and reviewed [41, 113–116]. The proposed mechanism has recently been confirmed with an electrospray ionization MS study (Scheme 5.58) [117]. It can be expected that organocatalysis will evolve into a mature field of catalysis within the next few years, adding many green approaches towards essential building blocks in organic chemistry.

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6
Hydrolysis

6.1
Introduction

Hydrolysis reactions are straightforward to carry out and they have a favorable equilibrium, since the solvent, i.e. water, is one of the reagents. This also has the advantage of being particularly green, since water is an environmentally benign solvent. However, alongside these advantages traditional hydrolysis reactions have many disadvantages. They are commonly performed with concentrated and thus corrosive acids or bases. These need to be neutralized at the end of the reaction, generating salts as waste. In addition high temperatures are often required, wasting much energy. If the substrate submitted to the hydrolysis reaction has more than one functional group lack of selectivity becomes a major problem; delicate structures might even be completely degraded. At the end of the reaction the product needs to be separated from the water, which is often difficult and energy consuming, especially if it involves a distillation. Furthermore hydrolysis reactions are never 100% atom efficient, depending on the group that is cleaved off they can even be quite atom inefficient. From a green point of view much can still be improved in traditional hydrolysis reactions [1]. Different approaches, such as solid acids and bases (Chapter 2), have been developed to address some of these problems. However, only one type of catalysis offers a solution to almost all of the aspects, namely: Biocatalysis.

Enzymes can hydrolyse esters, amides and nitriles under very mild, non-corrosive conditions at neutral pH values. Temperatures can be kept low, however, given the great thermostability of many enzymes, they can be raised if necessary. Due to the mild reaction conditions even fragile substrates can be converted selectively. Indeed, this is one of the major advantages of hydrolases (hydrolyzing enzymes). They are highly selective, allowing chemo-, regio-, and enantioselective hydrolyses of different functional groups in the substrates.

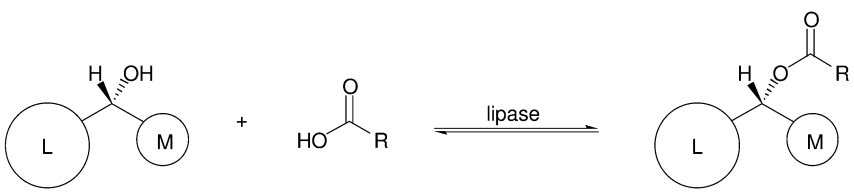
This chapter consequently focuses on the application of enzymes for the selective cleavage of esters, amides and nitriles [2]. Out of all the reported industrial applications of enzymes these type of hydrolyses constitute more than 40% [3]. Enzymatic hydrolyses are often performed because of the enantioselectivity of enzymes, and in particular of the lipases that are used for the production of enantiopure fine chemicals.

6.1.1 Stereoselectivity of Hydrolases

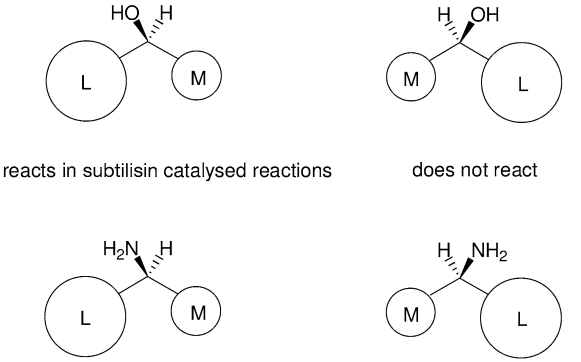
Hydrolases in general are enantioselective and in some cases even almost enantiospecific. Indeed, the great value of lipases, esterases, proteases, amidases and nitrilases for synthesis is, in part, due to their high enantioselectivity. While there are no general rules for all hydrolases, a brief description of the enantiopreferences of lipases and proteases (subtilisin) is given. Since lipases are widely used in hydrolytic kinetic resolutions of secondary alcohols their enantioselectivity is of particular interest. A model describing this has been established; it is known as Kazlauskas rule [2, 4–7]. This rule is based on the empirical observation that many lipases preferentially catalyse the conversion of one of the enantiomers of secondary alcohols. This holds true for both the synthesis and the hydrolysis reaction (Scheme 6.1). This stereoselectivity has been explained by the spatial arrangement of the catalytic residues on the basis of X-ray studies [8]. Interestingly, different lipases seem to have different methods for inducing chirality, even though they all work with a catalytic triad and the net result is the same [9, 10]. In the case of chiral primary amines Kazlauskas rule tends to give reliable results, too [11]. It is important to note that proteases (subtilisin) commonly show the opposite enantioselectivity (Scheme 6.2) [12]. Interestingly, the enantioselectivity of subtilisin can be solvent dependent and particularly in water the selectivities can be reversed [13]. Looking at all these types of hydrolases there is, in general, an enzyme available to hydrolyse either enantiomer of a secondary alcohol or chiral primary amine [2].

A general rule describing *Burkholderia cepacia* lipase (formerly called *Pseudomonas cepacia* lipase, PCL) catalysed conversions of primary alcohols with a chiral carbon in the β-position is depicted in Scheme 6.3. This rule, however, is only reliable if there are no oxygen substituents on the chiral carbon [14].

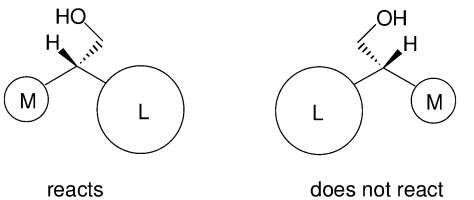
As well as the kinetic resolution of chiral alcohols, lipases can be employed to resolve chiral acids. Their enantioselectivity towards chiral acids or their esters is, however, less predictable. *Candida rugosa* lipase (CRL) has a general stereochemical preference for one enantiomer of acids with a chiral α-carbon (Scheme 6.4) [15].



Scheme 6.1 Enantioselectivity of lipases for secondary alcohols and their esters according to the rule of Kazlauskas: The faster reacting enantiomer of a secondary alcohol in most acylations or the faster reacting enantiomer of an ester in most hydrolyses is the enantiomer depicted. Most primary amines also follow this rule. L: largest substituent, M: medium-sized substituent.



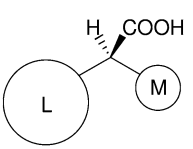
Scheme 6.2 Subtilisin commonly displays the opposite enantioselectivity to lipases. L: largest substituent, M: medium-sized substituent.



Scheme 6.3 PCL preferentially catalyses the hydrolysis/acylation of only one of the depicted enantiomers of the chiral primary alcohol (ester). The selectivity is low if oxygen is bound to the chiral carbon. L: largest substituent, M: medium-sized substituent.

The enantioselectivity that the enzymes display is not always very large. Indeed, there are even examples known for the opposite selectivity. Nonetheless, with these general rules, it is possible to address a large number of synthetic problems. For the detailed choice of the right biocatalyst for each particular substrate, several excellent reviews are available [2, 16–23].

For the other hydrolases general rules for their enantioselectivity are not available. This does not mean that their enantioselectivities are unknown; indeed for many enzymes much knowledge is available, however, less is known about the entire class of enzymes of which these individual, well-researched enzymes are an element.



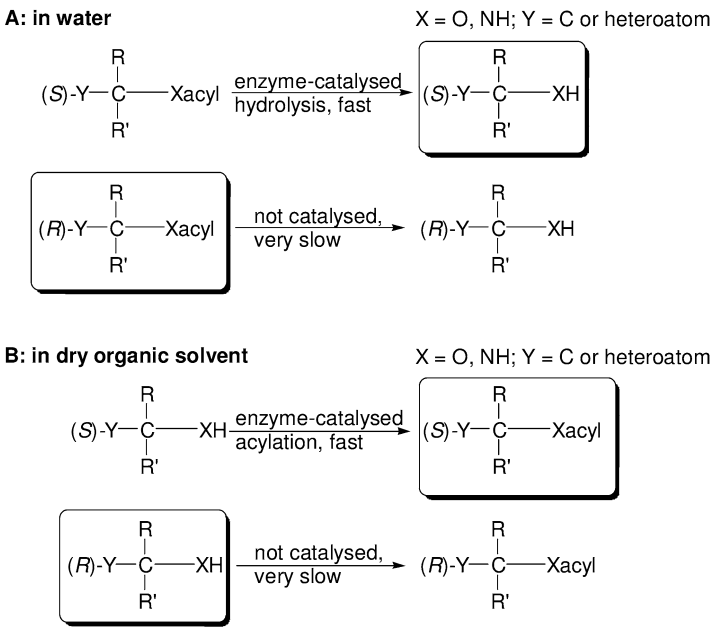
Scheme 6.4 Preferred enantiomer in CRL-catalysed hydrolysis and esterification reactions of chiral acids. L: largest substituent, M: medium-sized substituent.

6.1.2
Hydrolase-based Preparation of Enantiopure Compounds

Hydrolases catalyse the hydrolysis of esters, amides and nitriles under mild conditions. As mentioned above, they also display an often-remarkable enantioselectivity. This opens the opportunity to employ these enzymes for the preparation of enantiopure compounds. This desymmetrization has been reviewed [16–24] and different approaches [25–27] are possible.

6.1.2.1 **Kinetic Resolutions**

The most straightforward hydrolase-catalysed preparation of an enantiopure product from a racemic starting material is a kinetic resolution. In a kinetic resolution a racemic ester or amide is hydrolysed enantioselectively (Scheme 6.5 A). At the end of the reaction an enantiopure alcohol or amine is obtained. The unreactive enantiomer of the starting material should ideally also be enantiopure. Consequently the maximum yield for either compound in a kinetic resolution is only 50%. The enantiopurity of the products is dependent on the enantioselectivity of the enzyme; this is expressed as the enantiomeric ratio, E, of the enzyme [28]. If the E value for the enzyme is low (<25) neither the unreacted ester nor the obtained product are really pure at 50% conversion. Consequently kinetic re-



Scheme 6.5 A: Kinetic resolution of chiral esters or amides in water; B: kinetic resolution of chiral alcohols and amines in organic solvents.

solutions are only viable if the E of the enzyme is high (> 50) and even then 50% of the starting material ends up as waste and needs to be recycled.

Already in 1900 it was demonstrated that hydrolase-catalysed reactions are reversible [29]. By switching from water as a solvent to organic solvents and by employing activated acids as acyl donors the reactions can even be converted into irreversible acylations [2, 22, 27]. An essential step towards utilizing this potential of the hydrolases was their application in organic solvents [30, 31]. The solvents of choice are hydrophobic, so that the enzyme does not dissolve and deactivate, nor lose the often-essential water that is bound to it. Common organic solvents such as *tert*-butyl methyl ether (MTBE), toluene and hexane (which should be replaced by the less toxic heptane) have proven their value in this type of reaction [22, 27]. The replacement of water as a solvent and reactant with the alcohol commonly leads to enzyme deactivation and is not normally used. Based on these findings kinetic resolutions of chiral alcohols or amines can be performed in organic solvents (Scheme 6.5 B). Again a maximum yield of only 50% can be obtained. Additional disadvantages are that an excess of acyl donor needs to be added (2–4 equivalents) and that a potentially toxic solvent is used.

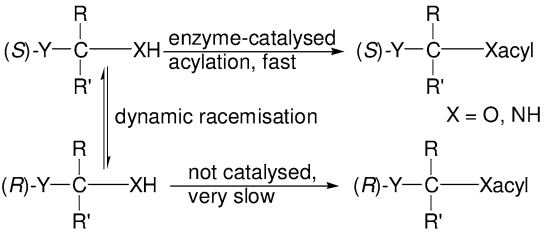
Of course the stereocenter can also be in the acid moiety, again kinetic resolutions can be performed either in water or in organic solvents. If the starting material is a chiral nitrile kinetic resolutions in water are equally possible, in that case a mixture of enantiopure nitrile and acid/amide are formed.

6.1.2.2 **Dynamic Kinetic Resolutions**

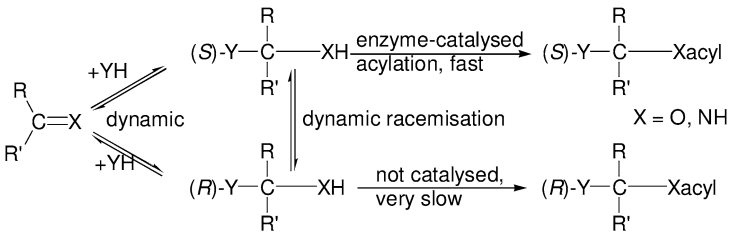
An elegant way to avoid the low yields and the need for recycling half of the material in the case of kinetic resolutions is a dynamic kinetic resolution (DKR). The dynamic stands for the dynamic equilibrium between the two enantiomers that are kinetically resolved (Scheme 6.6 A). This fast racemisation ensures that the enzyme is constantly confronted with an (almost) racemic substrate. At the end of the reaction an enantiopure compound is obtained in 100% yield from racemic starting material. Mathematical models describing this type of reaction have been published and applied to improve this important reaction [32, 33]. There are several examples, in which the reaction was performed in water (see below). In most cases the reaction is performed in organic solvents and the hydrolase-catalysed reaction is the irreversible formation of an ester (for example see Figs. 9.3, 9.4, 9.6, 9.12) or amide (for example see Figs. 9.13, 9.14, 9.16).

When looking at the above described dynamic kinetic resolution from a green point of view, then one thing can immediately be noticed: This reaction would be unnecessary if the starting material had been synthesized enantioselectively. A much more efficient way of performing a dynamic kinetic resolution is thus to start with a prochiral material. The reversible addition of another building block to this prochiral starting material is not only the formation of a new bond but at the same time a pathway for the rapid racemisation of the intermediate

A: starting with racemic substrate



B: starting with prochiral substrate



Scheme 6.6 A: Dynamic kinetic resolution of a racemic starting material yields 100% enantiopure product; B: in a synthetic dynamic kinetic resolution a new bond is formed enantioselectively with 100% yield.

racemic alcohol or amine. The irreversible hydrolase-catalysed reaction then induces the stereochemistry of the final product. Overall a new bond is formed enantioselectively (Scheme 6.6 B). This dynamic kinetic resolution is thus a truly synthetic reaction, indeed it is applied for the enantioselective synthesis of chiral α-hydroxy acids on an industrial scale (Schemes 5.16, 5.17) [34]. This type of dynamic kinetic resolution is also mainly performed in organic solvents (for examples see: Schemes 5.7 and 5.8 as well as Fig. 9.15).

6.1.2.3 **Kinetic Resolutions Combined with Inversions**

Another possibility to obtain 100% yield of the enantiopure product is to combine the kinetic resolution with an inversion reaction [25, 35, 36]. In this case an enzymatic hydrolysis is followed by a Mitsunobu inversion. It is, however, in fact a three-step reaction with solvent changes between the reactions. Similarly the sulfatase-catalysed enantioselective inversion of a racemic sulfate yields a homo-chiral mixture of alcohol and sulfate. This yields 100% enantiopure product after a second, acid-catalysed hydrolysis step, which is performed in organic solvent/water mixtures [26].

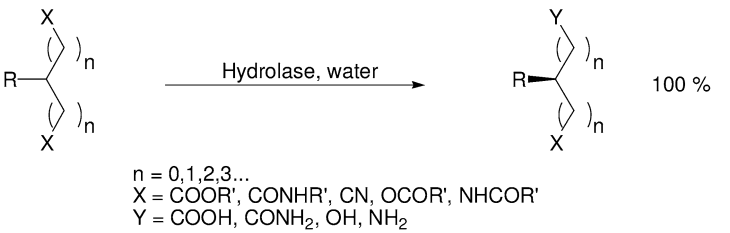
6.1.2.4 Hydrolysis of Symmetric Molecules and the "*meso*-trick"

Instead of starting with racemic starting material it is also possible to use symmetric substrates [25]. The hydrolase selectively catalyses the hydrolysis of just one of the two esters, amides or nitriles, generating an enantiopure product in 100% yield (Scheme 6.7). No recycling is necessary, nor need catalysts be combined, as in the dynamic kinetic resolutions, and no follow-up steps are required, as in the kinetic resolutions plus inversion sequences. Consequently this approach is popular in organic synthesis. Moreover, symmetric diols, diamines and (activated) diacids can be converted selectively into chiral mono-esters and mono-amides if the reaction is performed in dry organic solvents. This application of the reversed hydrolysis reaction expands the scope of this approach even further [22, 24, 27].

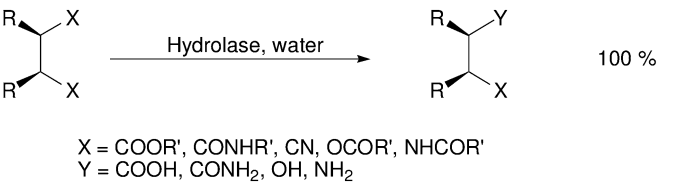
In the "*meso*-trick" the same principle is applied [25]. A symmetric compound, in this case a *meso* compound, is submitted to selective hydrolysis. The asymmetric compound that is generated in this manner is obtained in 100% yield and ideally high optical purity (Scheme 6.8). *Meso* compounds with diamino, diol or diacid functions can be converted to chiral mono-esters or mono-amides, too, if the reaction is performed in organic solvents [22, 24, 27].

6.2
Hydrolysis of Esters

The lipase or esterase-catalysed hydrolyses are straightforward and mild reactions. Consequently they can readily be performed on a large scale and they are suitable for first year undergraduate teaching [37]. Indeed, these enzymes are



Scheme 6.7 Hydrolases desymmetrise symmetrical compounds.



Scheme 6.8 The "*meso*-trick".

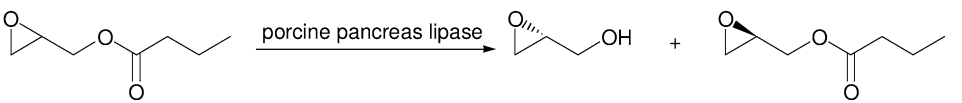
applied on an industrial scale, for the production of both fine and bulk chemicals [3, 34, 38–41].

6.2.1 Kinetic Resolutions of Esters

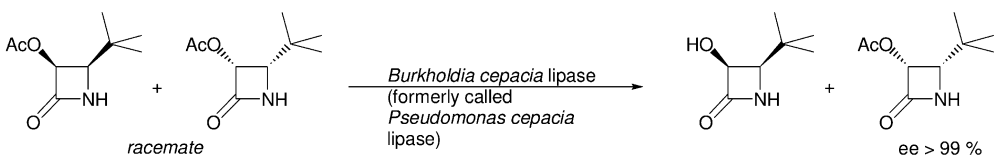
As early as 1984 the porcine pancreas lipase-catalysed enantioselective synthesis of (*R*)-glycidol was described. At pH 7.8 and ambient temperatures the reaction was allowed to proceed to 60% conversion (Scheme 6.9). This means that the enzyme was not extremely enantioselective, otherwise it would have stopped at 50% conversion. Nonetheless, after workup the (*R*)-glycidol was obtained in a yield of 45% with an *ee* of 92% [42]. This was a remarkable achievement and the process was developed into an industrial multi-ton synthesis by Andeno-DSM [34, 43]. While on the one hand a success story, it also demonstrated the shortcomings of a kinetic resolution. Most enzymes are not enantiospecific but enantioselective and thus conversions do not always stop at 50%, reactions need to be fine-tuned to get optimal *ee*s for the desired product [28]. As mentioned above kinetic resolutions only yield 50% of the product, the other enantiomer needs to be recycled. As a result of all these considerations this reaction is a big step forward but many steps remain to be done.

Scientists from Bristol-Myers Squibb developed a new side chain for Taxol, making it water-soluble. A kinetic resolution with *Pseudomonas cepacia* lipase (lipase PS-30 from Amano) was applied to obtain the desired material enantiopure (Scheme 6.10). After the lipase-catalysed hydrolysis of the wrong enantiomer (49% conversion) the ester was obtained with an *ee* of >99%. Separation and subsequent chemical cleavage of the ester yielded the desired enantiomer of the lactame, which could then be coupled to baccatin III [44].

The potential analgesic and replacements of the opiate-agonist tramadol, ε-hydroxyl-tramadol, was resolved enzymatically [43, 45]. Screening of several enzymes revealed that selective enzymes for both enantiomers were available. While pig liver esterase (PLE) hydrolysed the ester of the active enantiomer,



Scheme 6.9 The Andeno-DSM approach towards (*R*)-glycidol.



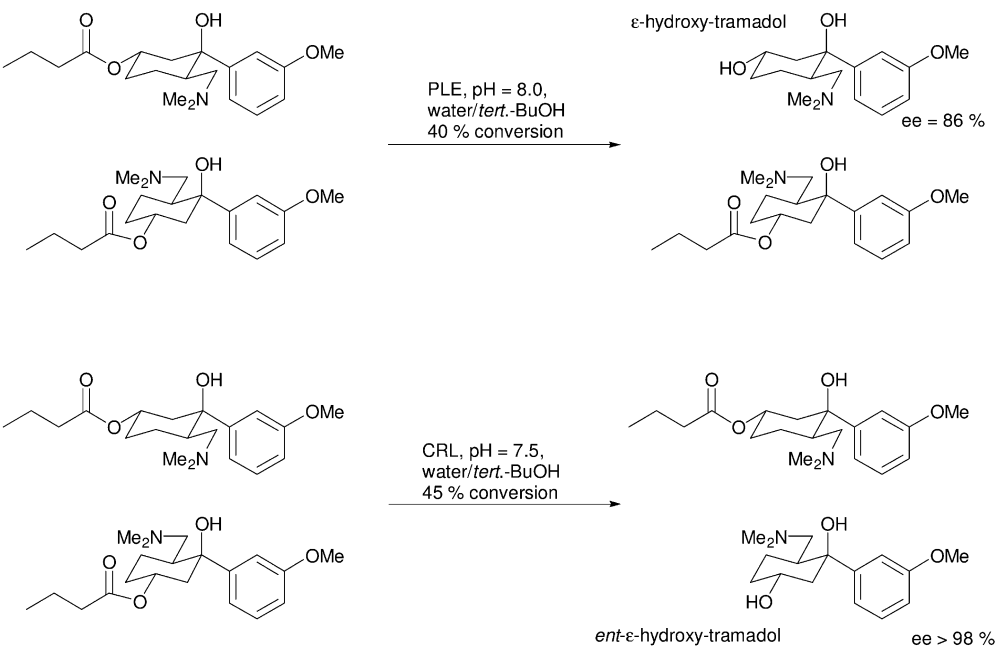
Scheme 6.10 Synthesis of a side-chain for an orally active taxane.

Candida rugosa lipase (CRL) hydrolysed the other enantiomer selectively (Scheme 6.11). This proves once again that in most cases enzymes with the desired stereoselectivity are available.

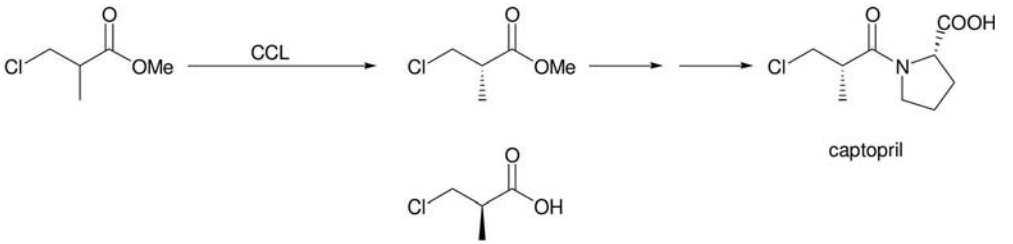
Utilising *Candida cylindracea* lipase (CCL) a chiral propionic acid was resolved by DSM [34, 46]. Only the undesired enantiomer of the ester was hydrolysed and at a conversion of 64% the remaining desired ester had an *ee* of 98% (Scheme 6.12). Although this means that the yield of the enantiopure ester is below 40% it did enable a new access to enantiopure captopril.

The atom efficiency of a kinetic resolution is increased if the starting material is not an ester but a lactone. Indeed, kinetic resolutions of lactones are used on an industrial scale. Fuji/Daiichi Chemicals produces D-pantothenic acid on a multi-ton scale based on such a resolution. D-Pantolactone is hydrolysed at pH 7 by a hydrolase from *Fusarium oxysporum* yielding D-pantoic acid with an *ee* of 96% while L-pantoic acid was barely detectable. The immobilized *Fusarium oxysporum* cells were recycled 180 times and retained 60% of their activity, demonstrating the great stability of this catalytic system [47–50].

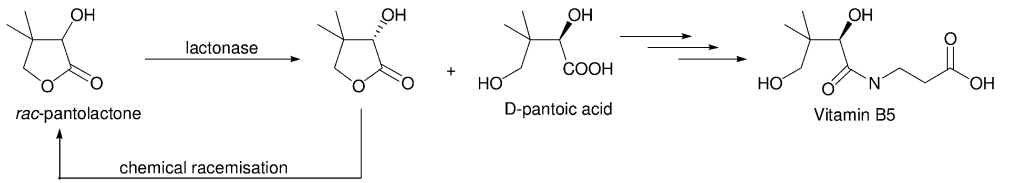
D-Pantoic acid is again lactonised and then converted into D-pantothenic acid, better known as vitamin B5 (Scheme 6.13). The remaining L-pantolactone can be racemised and recycled. Similar approaches based on L-specific lactonohydro-



Scheme 6.11 Depending on the enzyme chosen either enantiomer of ε-hydroxyl-tramadol can be hydrolysed enantioselectively.



Scheme 6.12 Enantioselective synthesis of captopril.



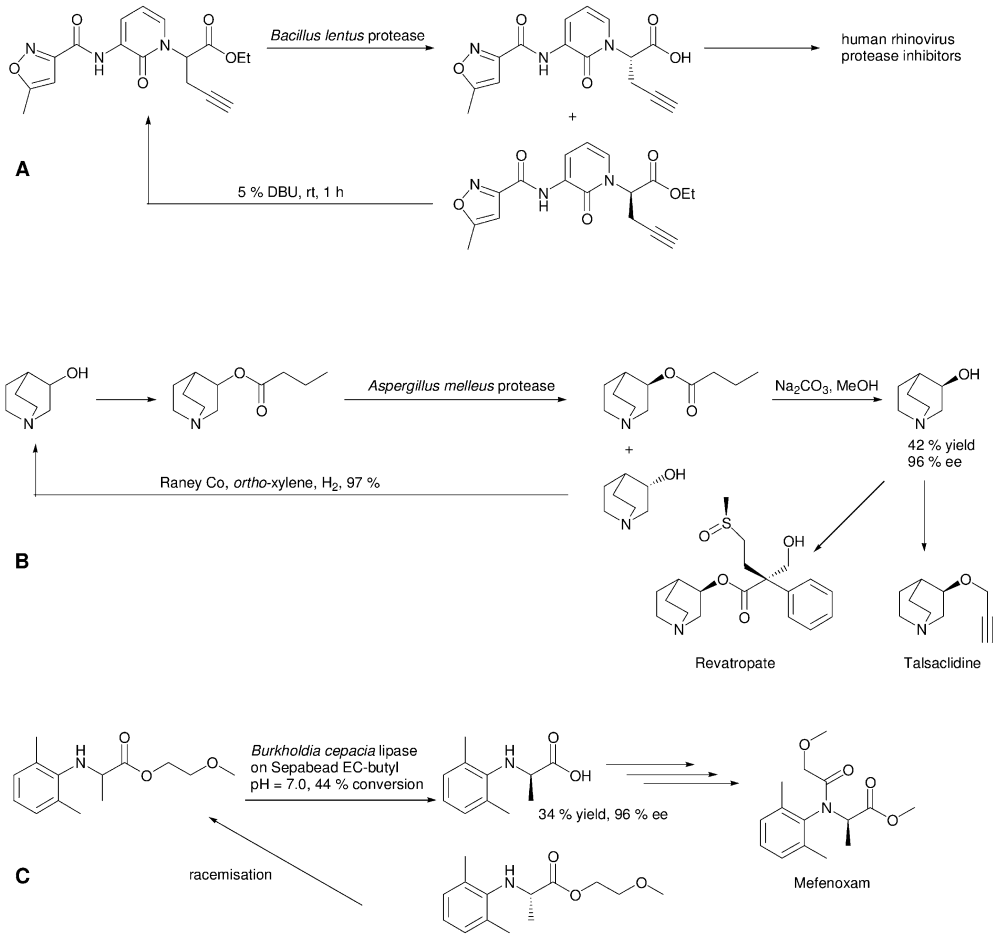
Scheme 6.13 Enantioselective synthesis of vitamin B5 based on a kinetic resolution with a lactonase.

lases have shown first success but are not yet commercialized [34, 51]. For alternative approaches toward vitamin B5 see also Chapter 8.

For the enantiopure production of human rhinovirus protease inhibitors, scientists from Pfizer developed a kinetic resolution and recycling sequence (Scheme 6.14 A). The undesired enantiomer of the ester is hydrolysed and can be racemised under mild conditions with DBU. This enzymatic kinetic resolution plus racemisation replaced a significantly more expensive chemical approach [52]. An enzymatic kinetic resolution, in combination with an efficient chemically catalysed racemisation, is the basis for a chiral building block for the synthesis of Talsaclidine and Revatropate, neuromodulators acting on cholinergic muscarinic receptors (Scheme 6.14 B). In this case a protease was the key to success [53]. Recently a kinetic resolution based on a *Burkholderia cepacia* lipase-catalysed reaction leading to the fungicide Mefenoxam was described [54]. Immobilisation of the enzyme ensured >20 cycles of use without loss of activity (Scheme 6.14 C).

6.2.2
Dynamic Kinetic Resolutions of Esters

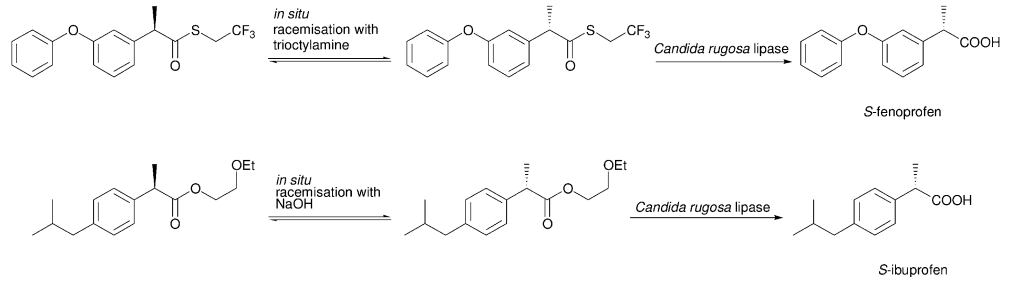
When the esters of chiral acids are submitted to a kinetic resolution, the wrong enantiomer cannot be racemised easily. However, if the chiral center of the acid can be racemised via the enol of the acid, this can be utilized. Esters do not enolize easily, but thioester and other activated esters [27] do so much more readily. This was exploited to convert kinetic resolutions into dynamic kinetic resolutions, thus increasing the yield from a maximum of 50% to a maximum of



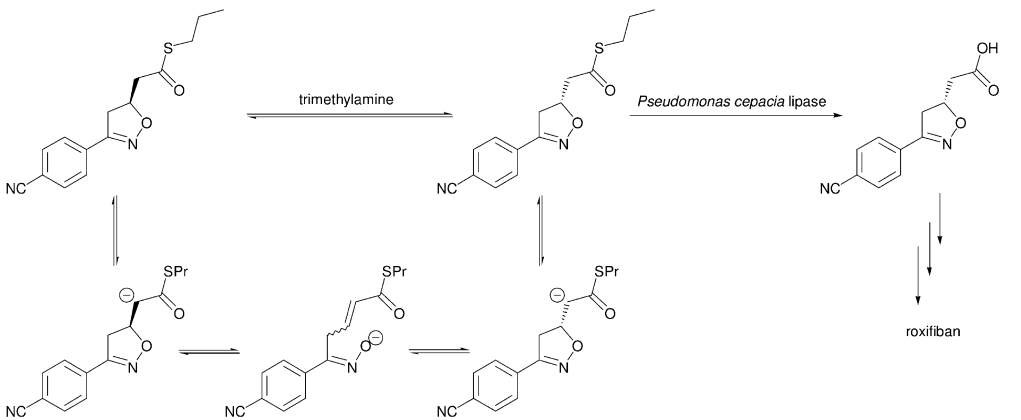
Scheme 6.14 A: Kinetic resolution and recycling of the wrong enantiomer for an intermediate of an antiviral drug; B: kinetic resolution and recycling of the wrong enantiomer for an intermediate of a neuromodulator; C: kinetic resolution and recycling of the wrong enantiomer for an intermediate of a fungicide.

100%. This approach was studied for naproxen trifluoroethylthioester [55], fenoprofen trifluoroethylthioester [56], naproxen trifluoroethylester [57] and ibuprofen 2-ethoxyethyl ester [58] (Scheme 6.15). Some of these reactions were not performed in water only, but in biphasic mixtures, due to solubility problems. This is a drawback from a green point of view, but the much higher yield and the fact that no recycling step is needed is a clear indication of the high efficiency of dynamic kinetic resolutions.

For the enantioselective preparation of roxifiban an entirely new approach for the *in situ* racemisation of the racemic starting material of the dynamic kinetic



Scheme 6.15 Dynamic kinetic resolutions via activated esters.

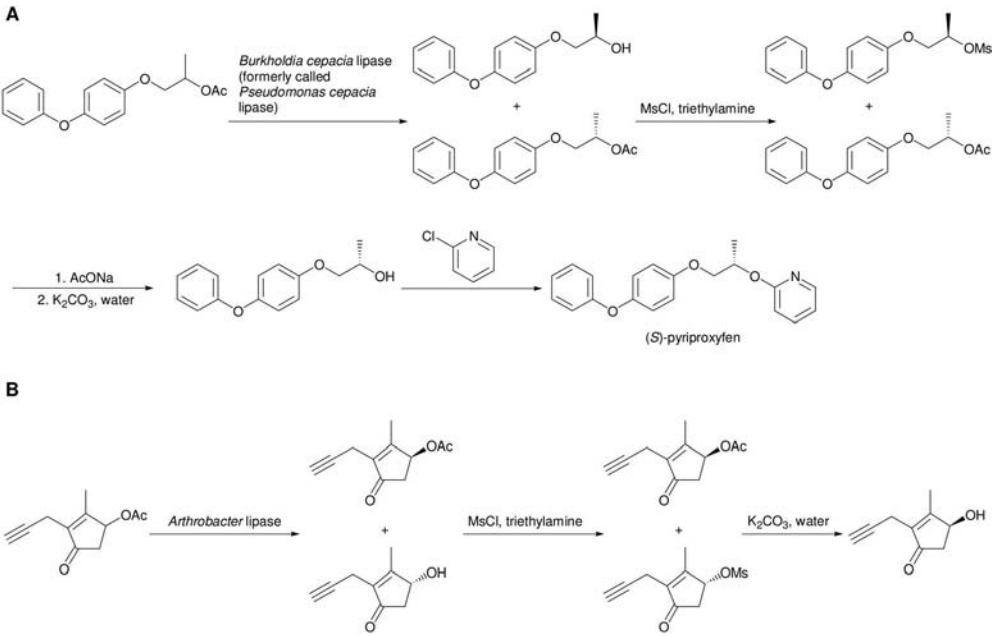


Scheme 6.16 Dynamic kinetic resolution of the key intermediate for the synthesis of roxifiban.

resolution was developed. Again the key to success was a thioester as activated ester. Instead of a racemisation of a stereocenter in the α-position, however, the base-catalysed racemisation occurred via a retro-Michael addition. Careful screening revealed that propylthiol was the thiol of choice, three equivalents of trimethylamine as a base should be used and that Amano PS-30 lipase catalyses the resolution step efficiently (Scheme 6.16). The overall procedure gave the acid in 80.4% yield with an *ee* of 94%. Recrystallisation improved this to >99.9% [59, 60].

6.2.3
Kinetic Resolutions of Esters Combined with Inversions

Scientists at Sumitomo developed this approach for the production of chiral insecticides [35, 61]. As discussed above, this approach involves several separate steps, an inversion replacing the racemisation that is normally necessary subsequent to a kinetic resolution. The advantage of this approach is that after the kinetic resolution no separation is necessary, since the reaction mixture is sub-



Scheme 6.17 A: Preparation of (*S*)-pyriproxyfen via a kinetic resolution–inversion sequence; B: chiral building blocks for the synthesis of pyrethroid insecticides are prepared via a kinetic resolution–inversion sequence.

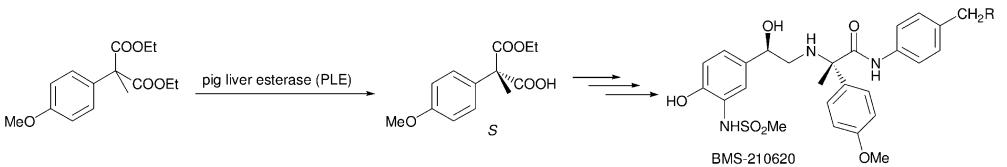
mitted to the inversion directly. A PCL-catalysed kinetic resolution was followed by the formation of a methanesulfonyl ester. The mixture of (*S*)-acetate and (*R*)-sulfonate was then treated with sodium acetate. A S_N2 reaction yielded pure (*S*)-acetate in theoretically 100% yield. Subsequent hydrolysis and treatment with 2-chloropyridine produced the insecticide (*S*)-pyriproxyfen (Scheme 6.17 A).

For the production of various pyrethroid insecticides the (*S*)-4-hydroxy-3-methyl-2-(2-propynyl)-2-cyclopenten-1-one was required. *Arthrobacter* lipase hydrolysed only the *R*-enantiomer of the racemic acetate and, after extraction, the mixture of the alcohol and acetate were submitted to methanesulfonyl chloride and triethylamine. The thus-obtained acetate/mesylate mixture was hydrolysed/inverted yielding 82% of (*S*)-4-hydroxy-3-methyl-2-(2-propynyl)-2-cyclopenten-1-one with an *ee* of 90% (Scheme 6.17 B). The procedure was also proved to work with other secondary alcohols and instead of the mesylate a Mitsunobu inversion could be applied [35].

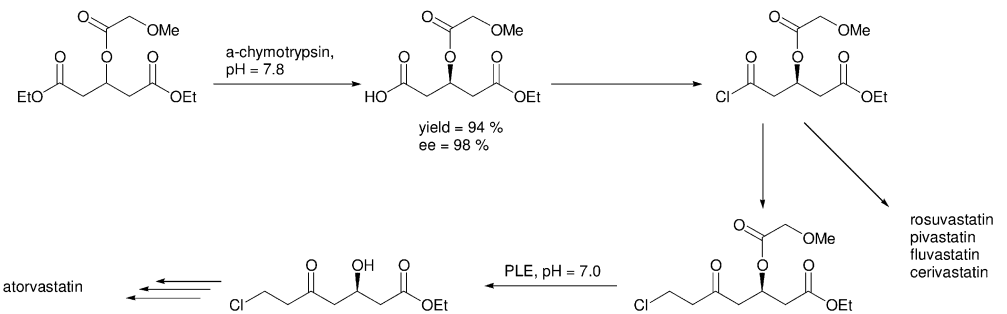
6.2.4
Hydrolysis of Symmetric Esters and the "*meso*-trick"

For a new potential β-3-receptor agonist a pig liver esterase-based enantioselective synthesis was devised (Scheme 6.18). The substituted malonic acid diester was hydrolysed at pH 7.2 and yielded 86% of the (*S*)-monoester with an *ee* of 97% [62]. This reaction immediately demonstrates the great advantage of starting with a symmetric molecule. The enzyme very efficiently desymmetrizes the diester and excellent yields with high optical purities are obtained. No extra steps are necessary and no additional chemicals need to be added.

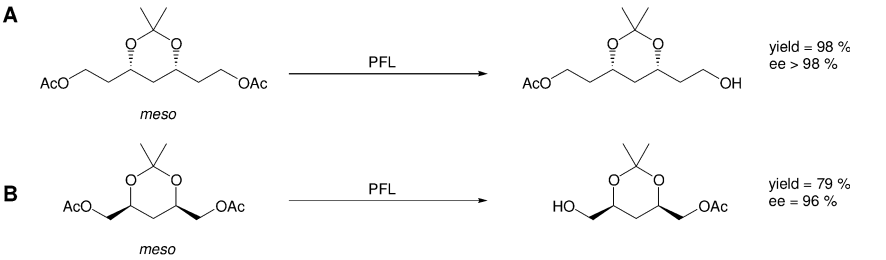
A similar strategy was chosen by the scientists from CIBA in their approach towards the statin side chain. Starting with a symmetric glutaric acid ester derivative, they succeeded in desymmetrising it with excellent yields and *ee* [63]. This hydrolysis is also remarkable since it was performed while the hydroxyl group was protected with an activated ester [27]. In the route towards atorvastatin this activated ester is selectively cleaved by PLE without hydrolyzing the remaining ethyl ester (Scheme 6.19). This once again demonstrates the great versatility of hydrolases in organic synthesis. A critical comparison of the different enzymatic routes towards the atorvastatin side chain was recently published, highlighting the power of enzymes for enantioselective synthesis [64].



Scheme 6.18 High yields and excellent optical purities are obtained in PLE-catalysed hydrolysis of an asymmetric starting material.



Scheme 6.19 Enantioselective CIBA route towards the statin side chains, based on a symmetric starting material.



Scheme 6.20 A and B: PFL-catalysed enantioselective hydrolysis of *meso*-compounds.

Symmetric starting materials have often been applied, not only in the form of symmetric diacids. The strategy has often been used to prepare chiral diols [65–67]; this has been reviewed very recently [68].

Chiral diols have also been prepared starting from *meso*-compounds [68–71]. Since *meso*-compounds are, in essence, symmetric molecules, the same applies as for the other symmetric starting materials. Indeed, this is exactly what was found: Even though the stereocenters of the protected heptane tetrol are far away from the ester groups that are to be hydrolysed stereoselectively, this is what happens [69, 70]. The high selectivity is partly due to the fact that the secondary alcohol groups are protected as a cyclic acetal, giving additional structural information to the enzyme (Scheme 6.20 A). A cyclic acetal also provides additional structural information in the enantioselective hydrolysis of a pentane tetrol derivative (Scheme 6.20 B) [71]. In both cases *Pseudomonas fluorescens* lipase (PFL) proved to be the enzyme of choice.

6.3 Hydrolysis of Amides

Due to the delocalization of electrons the amide group is normally planar and is significantly more stable than esters [72]. Nonetheless amides can be hydrolysed enzymatically under very mild conditions. Initially it might be expected that only enzymes that were evolved for this function by nature could hydrolyse this stable bond, but by now many examples are known where lipases and esterases hydrolyse amides, too [2, 34, 73]. A recent review discusses the mechanisms, modes of action and enantioselectivities of all the important enzymes [74].

The most prominent green example, the regioselective hydrolysis of an amide on an industrial scale, is the production of penicillin. PenG acylase selectively hydrolyses the more stable amide bond, leaving the β-lactam ring intact [75, 76]. For a full discussion of this example see Chapter 1 (Fig. 1.37) and Chapter 8. Since the starting material is already enantiopure the enzyme induces no stereoinformation. In other industrial processes the enantioselectivity of the enzymes is used. This is, in particular, the case in the production of natural and unnatural amino acids.

6.3.1 Production of Amino Acids by (Dynamic) Kinetic Resolution

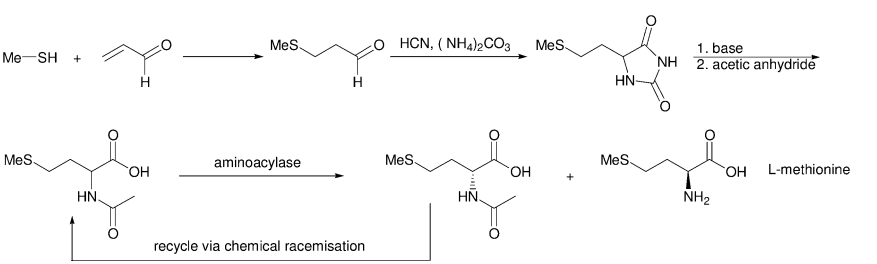
The industrial production of natural amino acids is mainly based on fermentation (Chapter 8), but not all 20 amino acids can be produced efficiently in this way [77]. For these amino acids other chemo-enzymatic approaches have been developed, several of them based on hydrolytic enzymes [78].

6.3.1.1 The Acylase Process

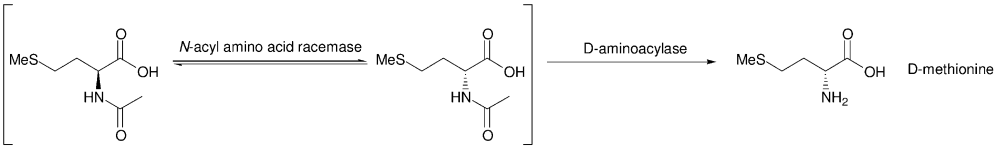
As early as 1969 an acylase was introduced for the industrial kinetic resolution of racemic *N*-acyl amino acids by Tanabe Seiyaku (Japan) [34, 78]. Later Degussa followed and developed similar biocatalytic processes [77–79]. The success of these industrial preparations of amino acids is based on the high selectivity and broad substrate specificity of acylase I [80]. In particular L-methionine is prepared in this way. The overall synthesis starts with a Michael addition of mercaptomethanol to acrolein, followed by a Strecker synthesis and ammonium carbonate addition to yield the hydantoin. Subsequent hydrolysis gives the racemic methionine in 95% yield calculated on the amount of acrolein used. Acylation results in the starting material for the enzyme reaction: *N*-acetylmethionine. Enantioselective hydrolysis with an acylase from *Aspergillus orycae* then releases the enantiopure L-methionine (Scheme 6.21) [77]. Overall the synthesis is a remarkable feat, acrolein is converted highly efficiently into racemic methionine and the final product is of food grade. However, the process has two drawbacks: the formation of the hydantoin that is later destroyed is not very atom efficient and the kinetic resolution wastes 50% of the product and causes a large recycle stream.

A straightforward approach to avoid low yields is to perform the reaction as a dynamic kinetic resolution. Racemisation can be achieved chemically [33] or enzymatically, indeed a number of *N*-acyl amino acid racemases have been described and it has been demonstrated that they could be employed together with the L-*N*-acyl amino acylase for the production of optically pure methionine [81].

The acylase process can also be applied for the production of D-amino acids. These amino acids are valuable building blocks in pharmaceutical chemistry and they can be prepared with high enantiopurity by the action of a D-*N*-acyl



Scheme 6.21 The Degussa synthesis of L-methionine.



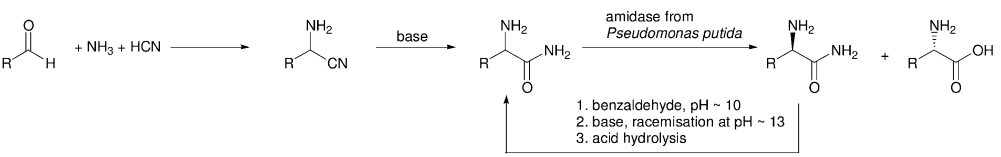
Scheme 6.22 Two-enzyme catalysed dynamic kinetic resolution for the preparation of D-amino acids.

amino acylase [82]. Again it is possible to combine the acylase reaction with a *N*-acyl amino acid racemase to obtain 100% product (Scheme 6.22) [81].

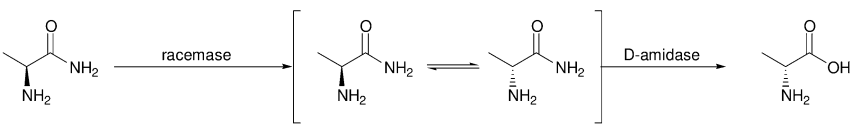
6.3.1.2 **The Amidase Process**

DSM developed a slightly different approach towards enantiopure amino acids. Instead of performing the Strecker synthesis with a complete hydrolysis of the nitrile to the acid it is stopped at the amide stage. Then a stereoselective amino acid amidase from *Pseudomonas putida* is employed for the enantioselective second hydrolysis step [83], yielding enantiopure amino acids [34, 77, 78]. Although the reaction is a kinetic resolution and thus the yields are never higher than 50% this approach is overall more efficient. No acylation step is necessary and the atom efficiency is thus much higher. A drawback is that the racemisation has to be performed via the Schiff's base of the D-amide (Scheme 6.23).

Recently it was reported that an α-amino-ε-caprolactam racemase from *Achromobacter obae* can racemise α-amino acid amides efficiently. In combination with a D-amino acid amidase from *Ochrobactrum anthropi* L-alanine amide could be converted into D-alanine. This tour de force demonstrates the power of the racemase [84]. If racemic amide is used as a starting material the application of this racemase in combination with a D- or L-amidase allows the preparation of 100% D- or L-amino acid, a dynamic kinetic resolution instead of DSM's kinetic resolution (Scheme 6.24).



Scheme 6.23 The DSM amidase process.

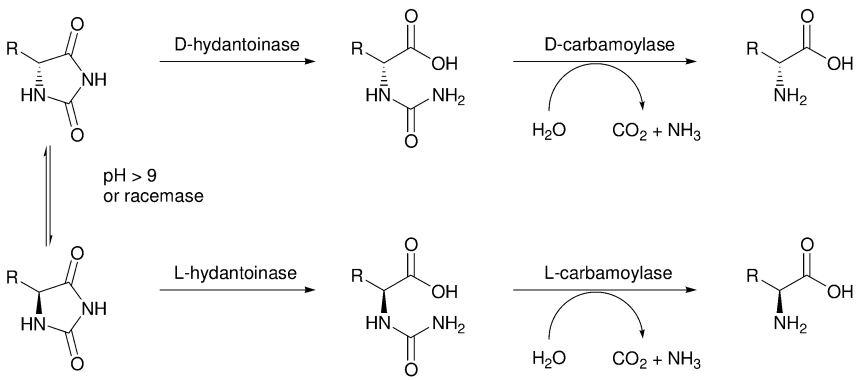


Scheme 6.24 α-Amino-ε-caprolactam racemase enables a dynamic kinetic resolution.

6.3.1.3 The Hydantoinase Process

The enzymes of the nucleic acid metabolism are used for several industrial processes. Related to the nucleobase metabolism is the breakdown of hydantoins. The application of these enzymes on a large scale has recently been reviewed [85]. The first step in the breakdown of hydantoins is the hydrolysis of the imide bond. Most of the hydantoinases that catalyse this step are D-selective and they accept many non-natural substrates [78, 86]. The removal of the carbamoyl group can also be catalysed by an enzyme: a carbamoylase. The D-selective carbamoylases show wide substrate specificity [85] and their stereoselectivity helps improving the overall enantioselectivity of the process [34, 78, 85]. Genetic modifications have made them industrially applicable [87]. Fortunately hydantoins racemise readily at pH > 8 and additionally several racemases are known that can catalyze this process [85, 88]. This means that the hydrolysis of hydantoins is always a dynamic kinetic resolution with yields of up to 100% (Scheme 6.25). Since most hydantoinases are D-selective the industrial application has so far concentrated on D-amino acids. Since 1995 Kaneka Corporation has produced ~2000 tons/year of D-*p*-hydroxyphenylglycine with a D-hydantoinase, a D-carbamoylase [87] and a base-catalysed racemisation [85, 89].

While the production of D-amino acids is well established the preparation of L-amino acids is difficult due to the limited selectivity and narrow substrate spectrum of L-hydantoinases. This can be circumvented by employing rather unselective hydantoinases in combination with very enantioselective L-carbamoylases and carbamoyl racemases [90]. Furthermore, a D-hydantoinase has been genetically modified and converted into a L-hydantoinase. This enzyme can be used on a 100-kg scale for the production of L-*tert*-leucine [34]. Finally, the fact that the X-ray structure of an L-hydantoinase is known gives hope that side-directed mutagenesis will lead to improved L-hydantoinases [91].



Scheme 6.25 The hydantoinase process for the production of amino acids.

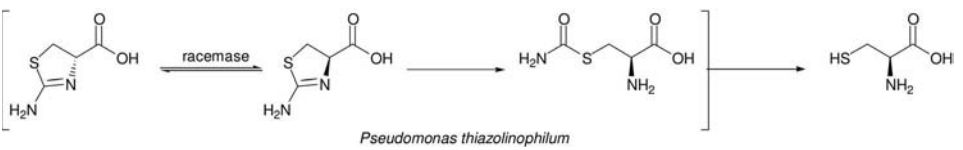
6.3.1.4 Cysteine

In 1978 the conversion of the racemic 2-amino-Δ^2-thiazoline-4-carboxylic acid into L-cysteine, catalysed by *Pseudomonas thiazolinophilum* cells, was reported [92]. Due to a racemase the overall process is a dynamic kinetic resolution and yields of 95% are obtained in the industrial process (Scheme 6.26) [34].

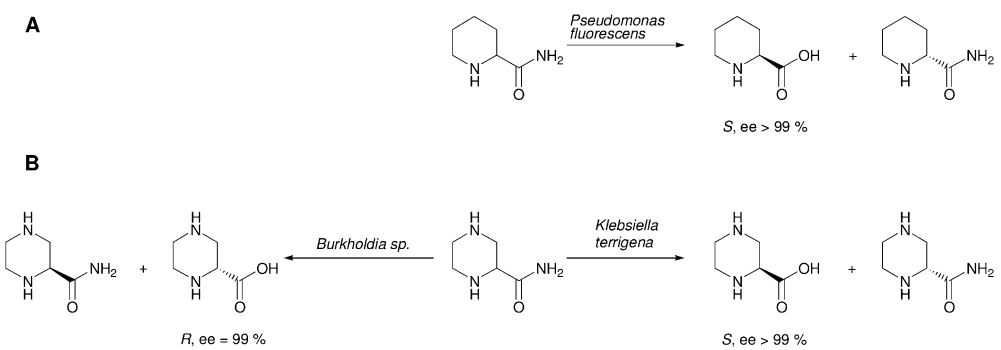
6.3.2 Enzyme-catalysed Hydrolysis of Amides

The hydrolysis of amides is not limited to the industrial synthesis of enantiopure amino acids. Lonza has developed routes towards (*S*)-pipecolic acid and (*R*)- and (*S*)-piperazine-2-carboxylic acid that are based on amidases [93, 94]. The processes are based on whole bacterial cells. In the case of the pipecolic acid, an important building block for pharmaceutical chemistry, an *S*-selective amidase in *Pseudomonas fluorescens* cells, catalyses the reaction with high selectivity and the acid is obtained with an *ee* > 99% (Scheme 6.27 A). For the preparation of piperazine-2-carboxylic acid from the racemic amide a *R*- and a *S*-selective amidase are available. Utilising *Klebsiella terrigena* cells the *S*-enantiomer is prepared with 42% isolated yield and *ee* > 99%, while *Burkholderia sp.* cells catalyse the formation of the *R*-enantiomer (*ee* = 99%, Scheme 6.27 B).

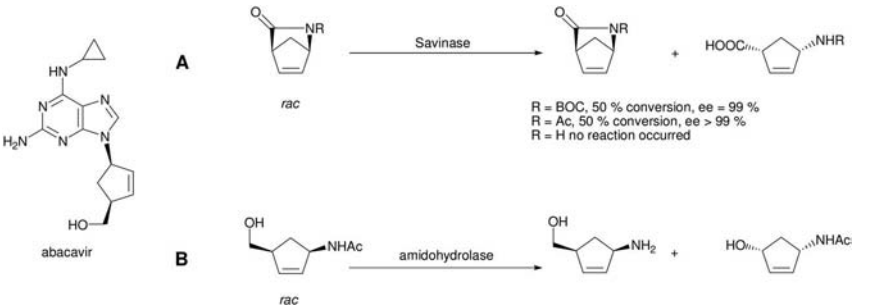
For the synthesis of the Glaxo anti-HIV drug abacavir (ziagen) a chiral cyclopentene derivative was needed. At Glaxo the application of savinase, a cheap en-



Scheme 6.26 Ajinomoto produces L-cysteine with 30 g l^{-1} via a *Pseudomonas thiazolinophilum*-catalysed process.



Scheme 6.27 Application of *R*- and *S*-selective amidases at Lonza for the production of pharmaceutical intermediates.

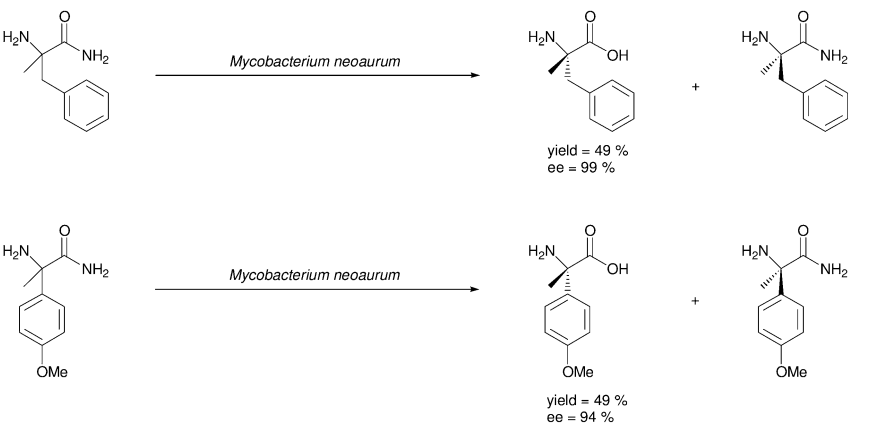


Scheme 6.28 A: the Glaxo route to enantiopure abacavir;
B: the Lonza route towards the chiral abacavir building block.

zyme that is used in bulk in the detergent industry, was chosen as the most suitable enzyme. Excellent conversions and enantioselectivities were obtained in the hydrolysis of the protected lactam (Scheme 6.28 A). Surprisingly the unprotected lactam was no substrate for the enzyme [95].

At Lonza [94] an alternative route was developed. Based on the acetylated amine a straightforward hydrolysis gave the desired amino alcohol (Scheme 6.28 B). The wrong enantiomer could not be recycled. Indeed, this is a problem with all the above-described kinetic resolutions: half of the starting material is waste and needs to be recycled.

At Bristol-Myers Squibb amidases were employed for the enantioselective preparation of new potential β-3-receptor agonists [62]. A kinetic resolution of the starting amides was achieved with very good to excellent enantioselectivities (Scheme 6.29). As already mentioned above, whole cells were used for these transformations.



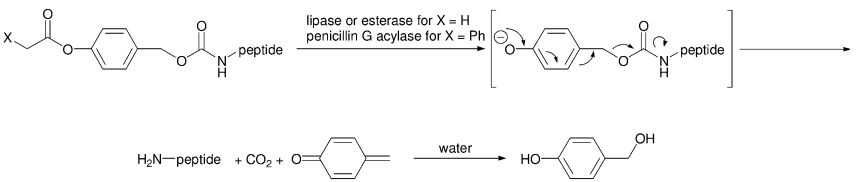
Scheme 6.29 Application of amidases at Bristol-Myers Squibb.

In general the hydrolysis of amides is performed as a kinetic resolution and not as a dynamic kinetic resolution. It is applied industrially [96] but in most cases the industrial kinetic resolution of amines, as performed for instance by BASF, is an acylation of racemic amines [38], rather than the hydrolysis of racemic amides. For the acylation of amines many different acyl donors [27] and enzymes can be used, including lipases (or a review see [97]).

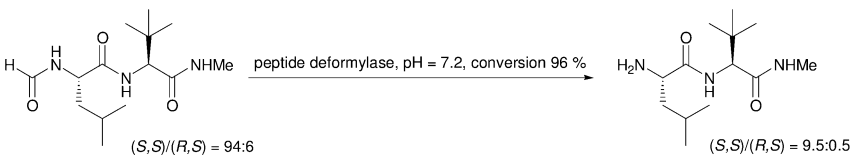
6.3.3
Enzyme-catalysed Deprotection of Amines

The use of protecting groups is one of the reasons why synthetic organic chemistry is far from being green and sustainable. However, it is also obvious that protection group chemistry cannot and will not disappear within the next few years, since the methodology to prepare complex structures without the use of protecting groups does not yet exist. An alternative is therefore to develop protecting groups, which can be cleaved mildly and selectively with the help of enzymes. A particularly elegant example is the variation of the Cbz- or Z-group [98]. By attaching a hydrolysable group in the para-position the Z-group can be split off. The enzyme used selectively cleaves the acetate or phenylacetate group, generating a phenolate ion in the para-position. This causes an immediate *in situ* fragmentation, releasing the amino-group. Overall an amino-group can thus be deprotected without the use of an amidase but with a lipase (Scheme 6.30). Consequently this deprotection technique can readily be applied in peptide synthesis without the risk of the enzymes degrading the peptide.

A class of enzymes that was made accessible only recently is the peptide deformylases. It was demonstrated that peptide deformylase can be used for kinetic resolutions, but they can also be employed to cleave off formyl protection groups [99]. Due to the stereoselectivity of the enzyme the enantiopurity of the product is also improved during the deprotection (Scheme 6.31).



Scheme 6.30 Enzymatic cleavage of a modified Z-protection group.



Scheme 6.31 Removal of a formyl protection group with a peptide deformylase.

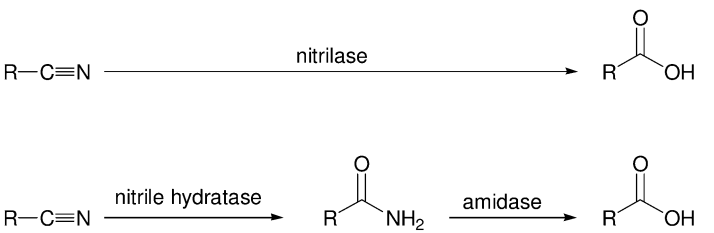
6.4
Hydrolysis of Nitriles

The nitrile group is a versatile building block, in particular since it can be converted into acids or amides. It undergoes hydrolysis but requires relatively harsh reaction conditions. Nature provides two enzymatic pathways for the hydrolysis of nitriles. The nitrilases convert nitriles directly into acids, while the nitrile hydratases release amides. These amides can then be hydrolysed by amidases (see also above). Often nitrile hydratases are combined with amidases in one host and a nitrile hydratase plus amidase activity can therefore be mistaken as the activity of a nitrilase (Scheme 6.32). A large variety of different nitrilases and nitrile hydratases are available [100, 101] and both types of enzyme have been used in industry [34, 38, 94].

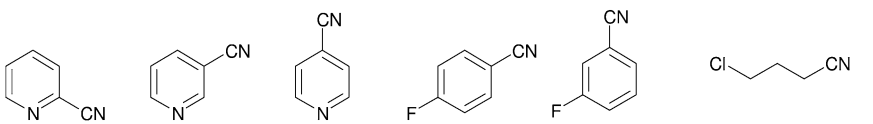
6.4.1
Nitrilases

The application of nitrilases is broad. A purified nitrilase from *Bacillus pallidus* was employed to hydrolyse a wide variety of aliphatic, aromatic and heteroaromatic nitriles and dinitriles (Scheme 6.33) [102]. Nitrilases have also been patented for the hydrolysis of α-substituted 4-methylthio-butyronitriles, however, no stereoselectivity was reported [103].

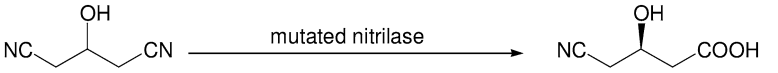
Although nitrilases do not always display high enantioselectivities [103] several examples of enantioselective nitrilases are known. Indeed they are used industrially for the synthesis of (*R*)-mandelic acid [34] and *S*-selective enzymes are also known [104]. In both cases the nitrilases were used for dynamic kinetic resolutions and they are discussed in Chapter 5 (Schemes 5.16 and 5.17).



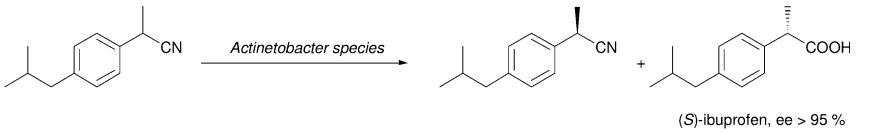
Scheme 6.32 Nitrile hydrolyzing enzymes.



Scheme 6.33 Good substrates for nitrilase from *Bacillus pallidus*.



Scheme 6.34 Diversa route to optically pure atorvastatin side-chain.



Scheme 6.35 Preparation of (*S*)-ibuprofen via a kinetic resolution.

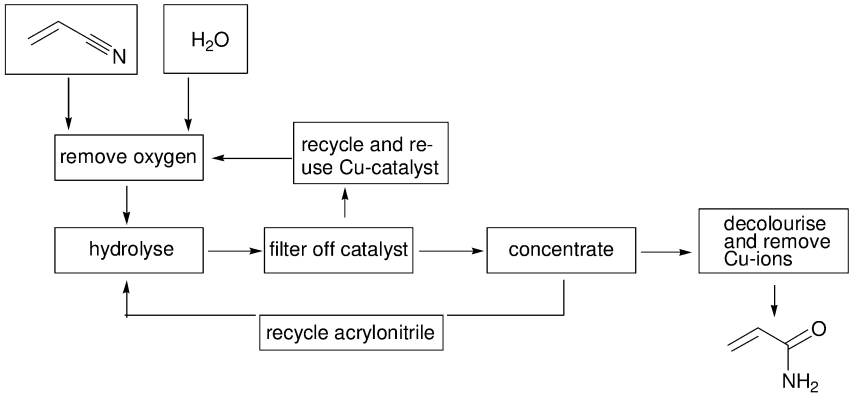
Nitrilases have been utilized successfully for the desymmetrisation of symmetric starting materials. At Diversa it was demonstrated that mutagenesis could create a highly selective nitrilase that was active at high substrate concentrations [105]. For their enzymatic route towards the atorvastatin (lipitor) side-chain this nitrilase now converts a symmetric precursor with $\sim 600\ g\ L^{-1}\ d^{-1}$ into the enantiopure (*R*)-4-cyano-3-hydroxybutyric acid (*ee* = 98.5%, Scheme 6.34).

Selective nitrilases have also been developed for the enantiopure preparation of ibuprofen [106]. In a kinetic resolution with *Acinetobacter sp.* AK226 (*S*)-ibuprofen could be prepared in good optical purity (Scheme 6.35).

6.4.2
Nitrile Hydratases

Nitrile hydratases are, just like nitrilases, versatile enzymes and their structure and application has been reviewed extensively [100, 101, 103, 106–108]. This is due to the great success of these enzymes on an industrial scale. Nitrile hydratases are employed not only in fine chemistry but also in bulk chemistry. The enzymatic production of acrylamide is a large-scale process that is replacing the traditional processes (see also Chapter 1, Fig. 1.42). Indeed this product has a history starting with homogeneous catalysis, moving to heterogeneous catalysis and ending at biocatalysis, demonstrating why biocatalysis is the catalysis type of choice in hydrolysis reactions. Acrylamide was first produced on a large scale in 1954 by American Cyanamid. The homogeneous approach is based on the sulfuric acid process (acrylonitrile : sulphuric acid : water in the ratio 1 : 1 : 1 at 60–80 °C, followed by cooling and neutralisation) and the product is purified by recrystallisation from benzene [109]. Huge amounts of salt are generated and benzene is definitely an outdated solvent. This stoichiometric process was improved and replaced by the heterogeneous process based on a Raney copper catalyst [109]. The conversion of acrylonitrile is >50% and the selectivity for the amide is close to 100% but the process is performed at 120 °C. In addition some copper leaches and needs to be removed at the end of the reaction (Scheme 6.36).

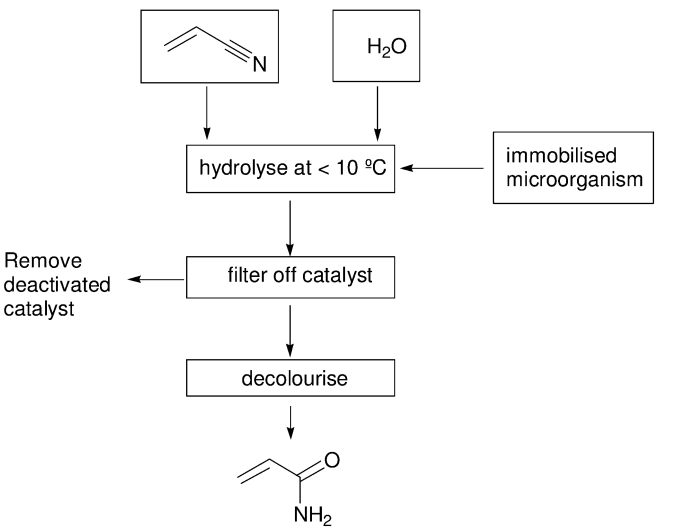
In contrast, the nitrile hydratase-based catalytic hydrolysis of acrylonitrile is an example of how green and sustainable chemistry should be performed [107–



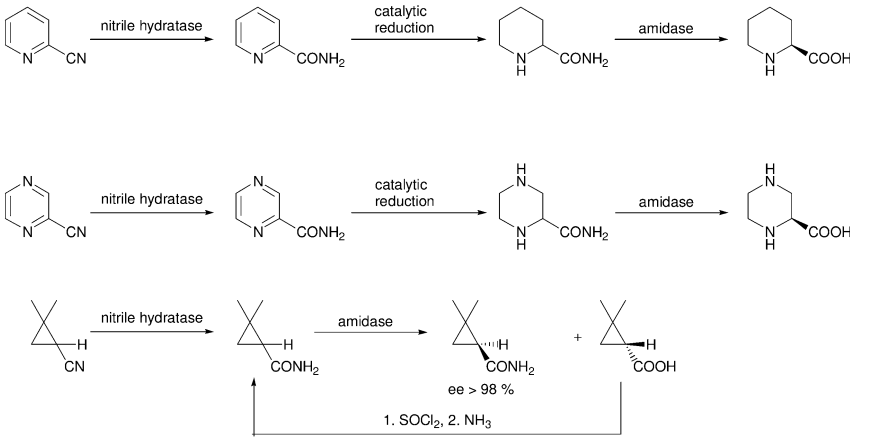
Scheme 6.36 Production of acrylamide with a Raney copper catalyst.

111]. Utilising an overexpressed nitrile hydratase from *Rhodococcus rhodochrous* in immobilised cells 30 000 tons per year are produced by Mitsubishi Rayon Co. Ltd. The selectivity of the nitrile hydratase is 99.99% for the amide, virtually no acrylic acid is detected and since all starting material is converted yields are excellent (>99%). The time space yield is 2 kg product per liter per day (Scheme 6.37). This clearly demonstrates the power of biocatalysis.

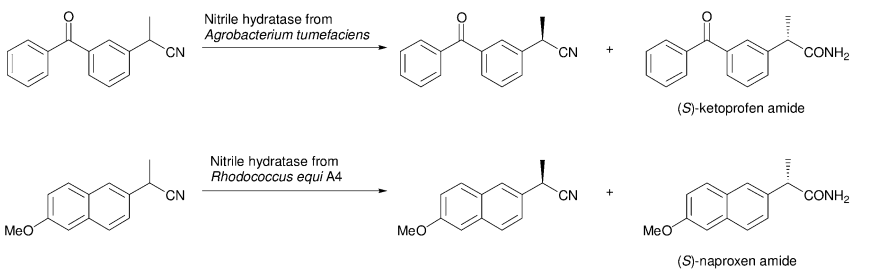
As mentioned in Chapter 1 the same *Rhodococcus rhodochrous* catalyses the last step in Lonza's >3500 tonnes/year nicotinamide synthesis [94, 111, 112]. Lonza has further developed this technology and currently synthesises a number of relevant fine chemical building blocks with nitrile hydratases [94, 113].



Scheme 6.37 Nitrile hydratase catalyses the selective formation of acrylamide.



Scheme 6.38 Nitrile hydratases play an essential role in the production of fine chemicals.



Scheme 6.39 Application of enantioselective nitrile hydratases for kinetic resolutions.

The nitrile hydrates employed are selective and stop at the amide stage. However, they display no relevant enantioselectivity. The enantioselectivity in the processes is always introduced by an amidase (see for instance Schemes 6.27 and 6.28) in a second hydrolysis step. Overall the syntheses are, remarkably, often purely catalytic and combine chemical catalysis for reductions with biocatalysis for hydrolyses and the introduction of stereoinformation (Scheme 6.38).

Although nitrile hydratases tend not to be stereoselective, examples of enantioselective enzymes are known [103, 106, 107, 114]. Of particular interest is the possibility to selectively hydrolyse 2-phenylproprionitriles, the core structure for ibuprofen and many other profens [103, 107, 114, 115]. This enables the enantioselective synthesis of the amides of ketoprofen and naproxen (Scheme 6.39).

6.5 Conclusion and Outlook

Biocatalysis is the answer to many problems in hydrolysis reactions. It enables the mild, selective and often enantioselective hydrolysis of many very different esters, amides and nitriles. Due to the very high selectivity of hydrolases a bulk chemical, acrylamide can now be produced in a clean and green manner, that is also more cost efficient than the chemical routes. The enantioselectivity of the hydrolases enables the production of commodity chemicals like enantiopure amino acids and also of many fine chemicals and drugs. A recent review summarizes the current state of the art for the biocatalytic synthesis of chiral pharmaceuticals [116], demonstrating the progress made. However, another recent review on the asymmetric synthesis of pharmaceuticals [117] reveals how much still needs to be done: less than five reactions in that review involve enzymes, most steps utilize stoichiometric reagents. Given the rapid development in biotechnology and the many new biocatalysts that have recently become available [118] and that will become available via the different genetic approaches [119, 120] it can be expected that the application of biocatalysis as described here is but a beginning.

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7
Catalysis in Novel Reaction Media

7.1
Introduction

7.1.1
Why Use a Solvent?

Organic reactions are generally performed in a solvent and there are several good reasons for this:

- Reactions proceed faster and more smoothly when the reactants are dissolved, because of diffusion. Although reactions in the solid state are known [1] they are often condensations in which a molecule of water is formed and reaction takes place in a thin film of water at the boundary of the two solid surfaces. Other examples include the formation of a liquid product from two solids, e.g. dimethylimidazolium chloride reacts with aluminum chloride to produce the ionic liquid, dimethylimidazolium tetrachloroaluminate [2]. It is worth noting, however, that not *all* of the reactant(s) have to be dissolved and reactions can often be readily performed with suspensions. Indeed, so-called solid-to-solid conversions, whereby a reactant is suspended in a solvent and the product precipitates, replacing the reactant, have become popular in enzymatic transformations [3]. In some cases, the solvent may be an excess of one of the reactants. In this case the reaction is often referred to as a solvolysis, or, when the reactant is water, hydrolysis.
- The solvent may have a positive effect on the rate and or selectivity of the reaction. The general rule is: reactions that involve ionic intermediates will be faster in polar solvents. For example, S_N1 substitutions proceed best in polar solvents while S_N2 substitutions, which involve a covalent intermediate, fare better in apolar solvents. The solvent may effect the position of an equilibrium, e.g. in a keto–enol mixture, thereby influencing selectivity in a reaction which involves competition between the two forms.
  Similarly, the solvent can have a dramatic effect on the rate and selectivity of catalytic reactions e.g. this is often the case in catalytic hydrogenations.
- The solvent acts as a heat transfer medium, that is it removes heat liberated in an exothermic reaction. It reduces thermal gradients in a reaction vessel, allowing a smooth and safe reaction. This is perfectly illustrated by the con-

cept of reflux, in which the reaction temperature is kept constant by allowing the solvent to boil and condense on a cold surface, before being returned to the reactor. In this way, a highly exothermic reaction can be prevented from 'running away'. Although reactions between two liquids obviously do not require a solvent, it is often prudent, for safety reasons, to employ a solvent (diluent).

7.1.2 Choice of Solvent

The choice of solvent depends on several factors. Obviously it should be liquid at the reaction temperature and, generally speaking, it is liquid at room temperature and below. Preferably it should be sufficiently volatile to be readily removed by simple distillation. Another important issue, in an industrial setting, is cost. Economic viability of the solvent will very much depend on the value of the product.

In the context of Green Chemistry, which we are primarily concerned with in this book, there are other major issues which have an important bearing on the choice of solvent. The solvent should be relatively nontoxic and relatively nonhazardous, e.g. not inflammable or corrosive. The word 'relatively' was chosen with care here, as Paracelsus remarked "the poison is in the dosage".

The solvent should also be contained, that is it should not be released to the environment. In recent years these have become overriding issues in the use of solvents in chemicals manufacture and in other industries. The FDA has issued guidelines for solvent use which can be found on the web site (www.fda.gov/cder/guidance/index.htm). Solvents are divided into four classes.

- Class 1 solvents should not be employed in the manufacture of drug substances because of their unacceptable toxicity or deleterious environmental effect. They include benzene and a variety of chlorinated hydrocarbons.
- Class 2 solvents should be limited in pharmaceutical processes because of their inherent toxicity and include more chlorinated hydrocarbons, such as dichloromethane, acetonitrile, dimethyl formamide and methanol.
- Class 3 solvents may be regarded as less toxic and of lower risk to human health. They include many lower alcohols, esters, ethers and ketones.
- Class 4 solvents, for which no adequate data were found, include di-isopropyl ether, methyltetrahydrofuran and isooctane.

Solvent use is being subjected to close scrutiny and increasingly stringent environmental legislation. Removal of residual solvent from products is usually achieved by evaporation or distillation and most popular solvents are, therefore, highly volatile. Spillage and evaporation inevitably lead to atmospheric pollution, a major environmental issue of global proportions. Moreover, worker exposure to volatile organic compounds (VOCs) is a serious health issue. Environmental legislation embodied in the Montreal (1987), Geneva (1991) and Kyoto (1997) protocols is aimed at strict control of VOC emissions and the eventual phasing

out of greenhouse gases and ozone depleting compounds. Many chlorinated hydrocarbon solvents have already been banned or are likely to be in the near future. Unfortunately, many of these solvents are exactly those that have otherwise desirable properties and are, therefore, widely popular for performing organic reactions. We all have experienced reactions that only seem to go well in a chlorinated hydrocarbon solvent, notably dichloromethane.

Another class of solvents which presents environmental problems comprises the polar aprotic solvents, such as dimethylformamide and dimethyl sulfoxide, that are the solvents of choice for, e.g. many nucleophilic substitutions. They are high boiling and not easily removed by distillation. They are also water-miscible which enables their separation by washing with water. Unfortunately, this leads inevitably to contaminated aqueous effluent.

These issues surrounding a wide range of volatile and nonvolatile, polar aprotic solvents have stimulated the fine chemical and pharmaceutical industries to seek more benign alternatives. There is a marked trend away from hydrocarbons and chlorinated hydrocarbons towards lower alcohols, esters and, in some cases, ethers. Diethyl ether is frowned upon because of its hazardous, inflammable nature, and tetrahydrofuran because of its water miscibility but higher boiling point, water immiscible ethers have become popular. Methyl *tert*-butyl ether (MTBE), for example, is popular and methyl tetrahydrofuran has recently been touted as an agreeable solvent [4]. Inexpensive natural products such as ethanol have the added advantage of being readily biodegradable and ethyl lactate, produced by combining two innocuous natural products, is currently being promoted as a solvent for chemical reactions.

It is worth noting that another contributing factor is the use of different solvents for the different steps in a multistep synthesis. Switching from one solvent to another inevitably leads to substantial wastage and chemists have a marked tendency to choose a different solvent for each step in a synthesis, But times are changing; as mentioned in Chapter 1, the new Pfizer process for sertraline uses ethanol for three consecutive steps, and ethyl acetate in the following step, obviating the need for the hexane, dichloromethane, tetrahydrofuran, and toluene used in the original process. The conclusion is clear: the problem with solvents is not so much their use but the seemingly inherent inefficiencies associated with their containment, recovery and reuse. Alternative solvents should therefore provide for their efficient removal from the product and reuse. The importance of alternative reaction media is underscored by the recent publication of an issue of Green Chemistry devoted to this topic [5].

The subject of alternative reaction media (neoteric solvents) also touches on another issue which is very relevant in the context of this book: recovery and reuse of the catalyst. This is desirable from both an environmental and an economic viewpoint (many of the catalysts used in fine chemicals manufacture contain highly expensive noble metals and/or (chiral) ligands.

7.1.3 Alternative Reaction Media and Multiphasic Systems

If a catalyst is an insoluble solid, that is, a heterogeneous catalyst, it can easily be separated by centrifugation or filtration. In contrast, if it is a homogeneous catalyst, dissolved in the reaction medium, this presents more of a problem. This offsets the major advantages of homogeneous catalysts, such as high activities and selectivities compared to their heterogeneous counterparts (see Table 7.1). However, as Blaser and Studer have pointed out [6], another solution is to develop a catalyst that, at least for economic reasons, does not need to be recycled.

Nonetheless, a serious shortcoming of homogeneous catalysis is the cumbersome separation of the (expensive) catalyst from reaction products and the quantitative recovery of the catalyst in an active form. Separation by distillation of reaction products from catalyst generally leads to heavy ends which remain in the catalyst phase and eventually deactivate it. In the manufacture of pharmaceuticals quantitative separation of the catalyst is important in order to avoid contamination of the product. Consequently there have been many attempts to heterogenize homogeneous catalysts by attachment to organic or inorganic supports or by separation of the products from the catalyst using a semipermeable membrane. However, these approaches have, generally speaking, not resulted in commercially viable processes, for a number of reasons, such as leaching of the metal, poor catalyst productivities, irreproducible activities and selectivities and degradation of the support.

This need for efficient separation of product and catalyst, while maintaining the advantages of a homogeneous catalyst, has led to the concept of liquid–liquid biphasic catalysis, whereby the catalyst is dissolved in one phase and the reactants and product(s) in the second liquid phase. The catalyst is recovered and recycled by simple phase separation. Preferably, the catalyst solution remains in the reactor and is reused with a fresh batch of reactants without further treatment or, ideally, it is adapted to continuous operation. Obviously, both solvents are subject to the same restrictions as discussed above for monophasic systems. The biphasic concept comes in many forms and they have been summarized by Keim in a recent review [7]:

Table 7.1 Homogeneous vs Heterogeneous Catalysis

	Homogeneous	**Heterogeneous**
Advantages	– Mild reaction conditions – High activity & selectivity – Efficient heat transfer	– Facile separation of catalyst and products – Continuous processing
Disadvantages	– Cumbersome separation & recycling of catalyst – Not readily adapted to a continuous process	– Heat transfer problems – Low activity and/or selectivity

- Two immiscible organic solvents
- Water (aqueous biphasic)
- Fluorous solvents (fluorous biphasic)
- Ionic liquids
- Supercritical CO_2

7.2
Two Immiscible Organic Solvents

Probably the first example of a process employing the biphasic concept is the Shell process for ethylene oligomerization in which the nickel catalyst and the ethylene reactant are dissolved in 1,4-butanediol, while the product, a mixture of linear alpha olefins, is insoluble and separates as a second (upper) liquid phase (see Fig. 7.1). This is the first step in the Shell Higher Olefins Process (SHOP), the largest single feed application of homogeneous catalysis [7].

Because of the resemblance to the 1,4-butane diol in the above example, it is worth mentioning that poly(ethylene glycol) (PEG) and poly(propyleneglycol) (PPG) have attracted interest as novel solvents for catalytic processes (see Fig. 7.2 for examples). They are both relatively inexpensive and readily available materials. They are essentially non-toxic (PPG is often used as a solvent for pharmaceutical and cosmetic preparations and both are approved for use in beverages) and have good biodegradability. Moreover, they are immiscible with water, non-volatile, thermally robust and can, in principle, be readily recycled after removal of the product.

For example, PEG-200 and PEG-400 (the number refers to the average molecular weight) were used as solvents for the aerobic oxidation of benzylic alcohols catalyzed by the polyoxometalate, $H_5PV_2Mo_{10}O_{40}$ [8]. Combination of the same polyoxometalate with Pd(II) was used to catalyze the Wacker oxidation of propyl-

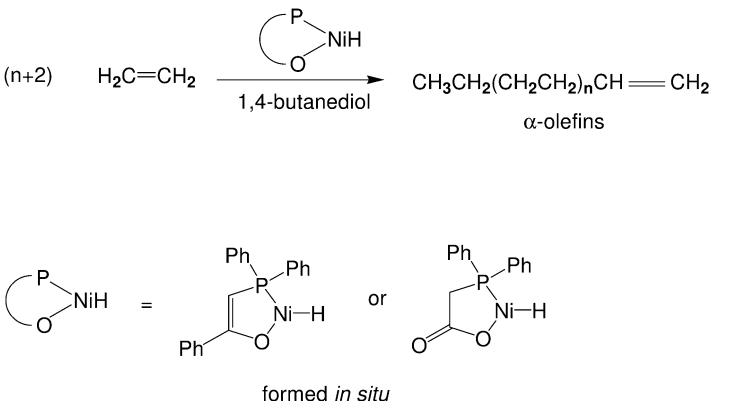


Fig. 7.1 The oligomerization step of the SHOP process.

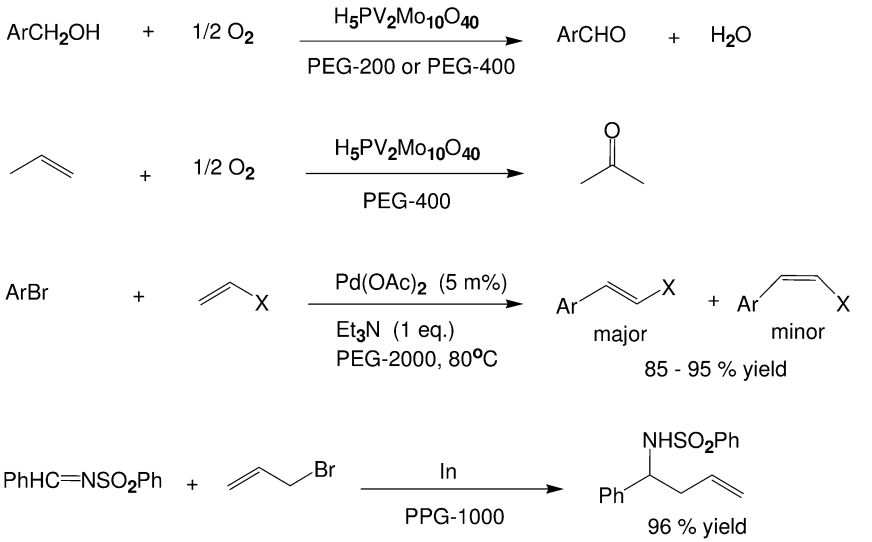


Fig. 7.2 Catalytic reactions in PEG or PPG.

ene to acetone [8, 9]. Similarly, PEG-400 was used as a reusable solvent for the Heck reaction [10], and PPG-1000 for the indium mediated synthesis of homoallylic amines from imines [11].

In the following sections we shall address the other forms of biphasic catalysis.

7.3 Aqueous Biphasic Catalysis

Water has several attractive features as a solvent and, as we have said elsewhere, the best solvent is no solvent, but if one has to use a solvent then let it be water. Water is the most abundant molecule on the planet and is, hence, readily available and inexpensive. It is nonflammable and incombustible and odorless and colorless (making contamination easy to spot). It has a high thermal conductivity, heat capacity and heat of evaporation, which means that exothermic reactions can be controlled effectively. It readily separates from organic solvents owing to its polarity, density and because of the hydrophobic effect [12], which makes it eminently suitable for biphasic catalysis. Indeed, water forms biphasic systems with many organic solvents, with fluorous solvents, some ionic liquids and with $scCO_2$ [13].

There are some disadvantages, however. Many organic compounds do not dissolve in water. Although the product should have a very low solubility in water the reactants must have some solubility since the catalyst is in the water phase. Reactants and or catalyst may not be stable or may be deactivated in water. If

the water is eventually contaminated it may be difficult to purify. Hence, the amount of water used should be kept to a minimum and recycled, as a catalyst solution, as many times as possible before eventual clean-up is required owing to accumulation of impurities.

The numerous benefits of aqueous biphasic catalysis clearly outweigh any disadvantages and, consequently, it is the most studied and most widely applied biphasic technology. Almost all reactions involving organometallic catalysis [14–25], including asymmetric syntheses [26] and polymerizations [27] have been performed in this way. A comprehensive coverage of all these examples goes beyond the scope of this book and the reader is referred to the numerous books and reviews on the subject [14–27] for more details. In this section we shall cover some salient examples which particularly illustrate the industrial utility and the greenness aspects of the methodology.

A prerequisite for aqueous biphasic catalysis is that the catalyst should be soluble in water and this is generally achieved by incorporating hydrophilic moieties in the ligand(s). Literally hundreds of water soluble ligands have been designed for use in aqueous biphasic catalysis. A few examples are shown in Fig. 7.3 and the reader is referred to a review [17] on the subject for more examples. By far the most commonly used water soluble ligands are the sulfonated phosphines. The first water soluble, sulfonated phosphine, $Ph_2P(C_6H_4\text{-}3\text{-}SO_3Na)$ (tppms), was prepared in 1958 by Chatt and coworkers, by sulfonation of triphenyl phosphine [28]. The solubility of tppms in water is 80 g L^{-1} at 20 °C.

Pioneering studies of the use of water soluble noble metal complexes of sulfonated phosphines as catalysts in aqueous biphasic systems were performed in the early 1970s, by Joo and Beck in Hungary and Kuntz at Rhone-Poulenc in France. Joo and Beck studied catalytic hydrogenations and transfer hydrogenations using Rh or Ru complexes of tppms [24]. Kuntz, on the other hand, pre-

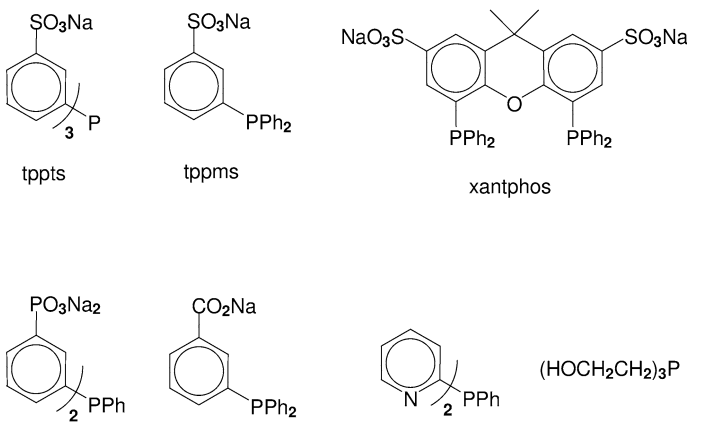


Fig. 7.3 Water soluble phosphines.

pared trisulfonated triphenylphosphine (tppts), by using longer sulfonation times, and it proved to be highly soluble in water (1100 g L^{-1} at 20 °C).

7.3.1 Olefin Hydroformylation

Kuntz subsequently showed that the RhCl $(tppts)_3$ catalyzed the hydroformylation of propylene in an aqueous biphasic system [29]. These results were further developed, in collaboration with Ruhrchemie, to become what is known as the Ruhrchemie/Rhone-Poulenc two-phase process for the hydroformylation of propylene to n-butanal [18, 19, 22, 30]. Ruhrchemie developed a method for the large scale production of tppts by sulfonation of triphenylphosphine with 30% oleum at 20 °C for 24 h. The product is obtained in 95% purity by dilution with water, extraction with a water insoluble amine, such as tri(isooctylamine), and pH-controlled re-extraction of the sodium salt of tppts into water with a 5% aqueous solution of NaOH. The first commercial plant came on stream in 1984, with a capacity of 100 000 tons per annum of butanal. Today the capacity is ca. 400 000 tpa and a cumulative production of millions of tons. Typical reaction conditions are $T = 120$ °C, $P = 50$ bar, $CO/H_2 = 1.01$, tppts/Rh = 50–100, [Rh] = 10–1000 ppm. The $RhH(CO)(tppts)_3$ catalyst is prepared *in situ* from e.g. rhodium 2-ethylhexanoate and tppts in water.

The RCH/RP process (see Fig. 7.4) affords butanals in 99% selectivity with a n/i ratio of 96/4. Rhodium carry-over into the organic phase is at the ppb level. The process has substantial economic and environmental benefits compared with conventional processes for the hydroformylation of propylene using Rh or Co complexes in an organic medium [31]:

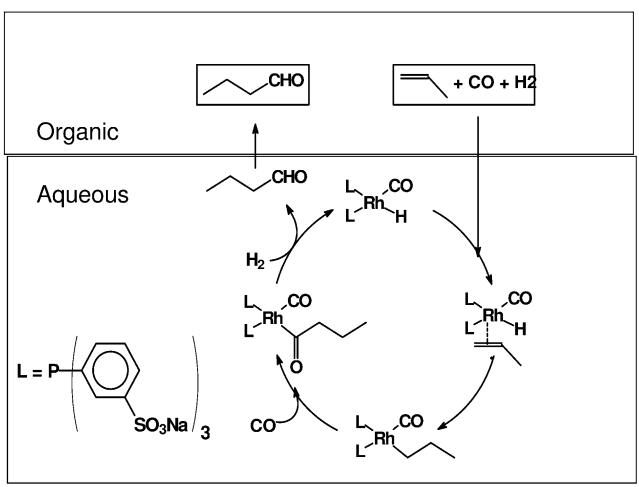


Fig. 7.4 RCH/RP two-phase oxo process.

- High catalyst activity and selectivity (less alkane production)
- High linearity (n/i) is obtained at lower ligand/Rh ratios
- Easy and essentially complete catalyst recovery
- Much simpler process engineering and the process is highly energy efficient (the plant is a net producer of heat)
- Virtual elimination of plant emissions and the avoidance of an organic solvent
- Conditions are less severe and heavy end formation is much less than in conventional processes where the product is separated from the catalyst by distillation. Moreover, the small amount of heavy ends (0.4%) formed is dissolved in the organic phase where it does not contaminate/deactivate the catalyst.

Based on the success of the RCH/RP process much effort was devoted to the development of new ligands that are even more efficient (see Fig. 7.5 for examples) [17]. However, to our knowledge they have not been commercialized, probably because they do not have a favorable cost/benefit ratio.

The aqueous biphasic hydroformylation concept is ineffective with higher olefins owing to mass transfer limitations posed by their low solubility in water. Several strategies have been employed to circumvent this problem [22], e.g. by conducting the reaction in a monophasic system using a tetraalkylammonium salt of tppts as the ligand, followed by separation of the catalyst by extraction into water. Alternatively, one can employ a different biphasic system such as a fluorous biphasic system or an ionic liquid/$scCO_2$ mixture (see later).

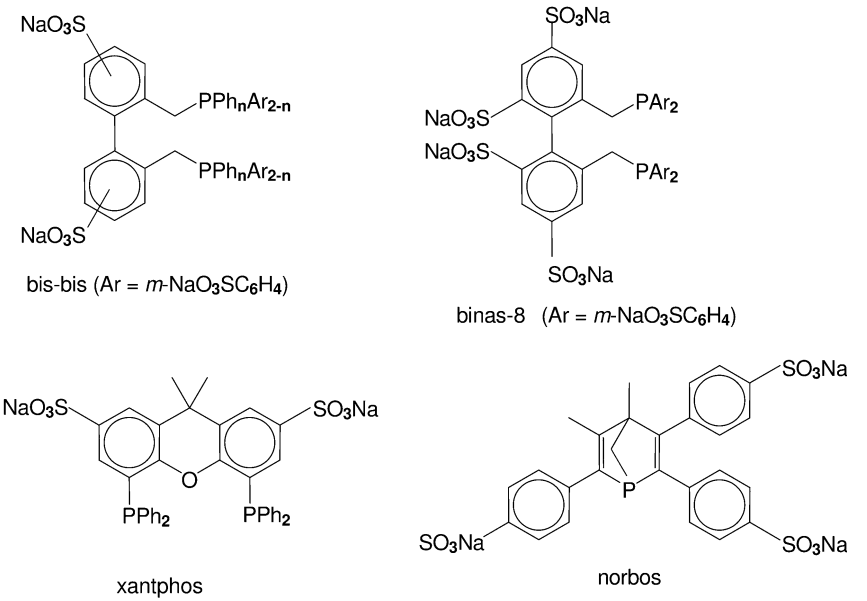


Fig. 7.5 Water soluble phosphine ligands for hydroformylation.

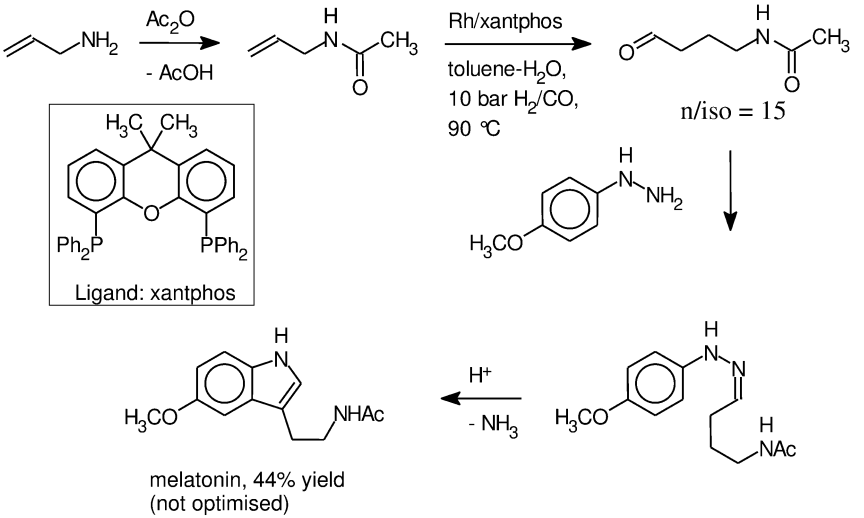


Fig. 7.6 Synthesis of melatonin using inverse aqueous phase catalysis.

What about when the substrate and product are water soluble? The problem of catalyst recovery in this case can be solved by employing *inverse aqueous biphasic catalysis*. An example is the hydroformylation of *N*-allylacetamide in an aqueous biphasic system in which the catalyst is dissolved in the organic phase and the substrate and product remain in the water phase. This formed the basis for an elegant synthesis of the natural product, melatonin, in which the aqueous solution of the hydroformylation product was used in the next step without work-up (Fig. 7.6) [32].

The success of aqueous biphasic hydroformylation stimulated a flurry of activity in the application of the concept to other reactions involving organometallic catalysis [14–27].

7.3.2 Hydrogenation

Following the pioneering work of Joo [24], the aqueous biphasic concept has been widely applied to catalytic hydrogenations and hydrogen transfer processes, using mainly complexes of Rh and Ru [15, 24, 25]. For example, Mercier et al., at Rhone-Poulenc, showed that hydrogenation of the unsaturated aldehyde, 3-methyl-2-buten-1-al, in the presence of Rh/tppts afforded the corresponding saturated aldehyde, while with Ru/tppts as the catalyst the unsaturated alcohol was obtained in high selectivity (Fig. 7.7) [33, 34]. Joo subsequently showed, using $RuCl_2(tppms)_2$ as the catalyst, that the chemoselectivity is pH dependent [35]. The active catalytic species for olefinic double bond hydrogenation is $RuHCl(tppms)_2$, formed in acidic solutions by dissociation of $RuHCl(tppms)_3$. In contrast, at basic pH the major species is $RuH_2(tppms)_4$, which catalyzes

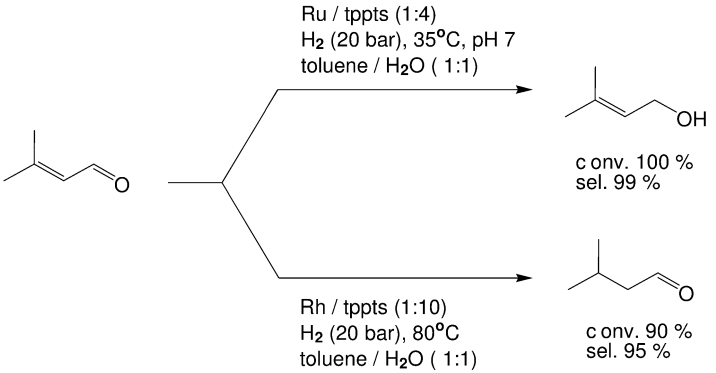


Fig. 7.7 Chemoselective hydrogenation of an unsaturated aldehyde.

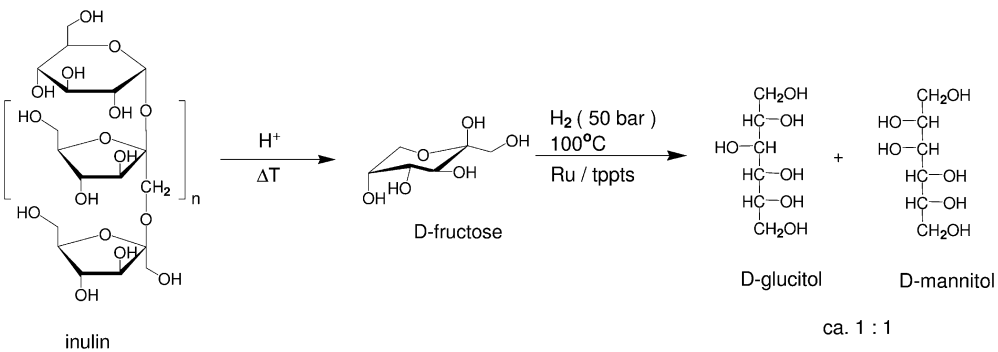


Fig. 7.8 Hydrogenation of carbohydrates in water.

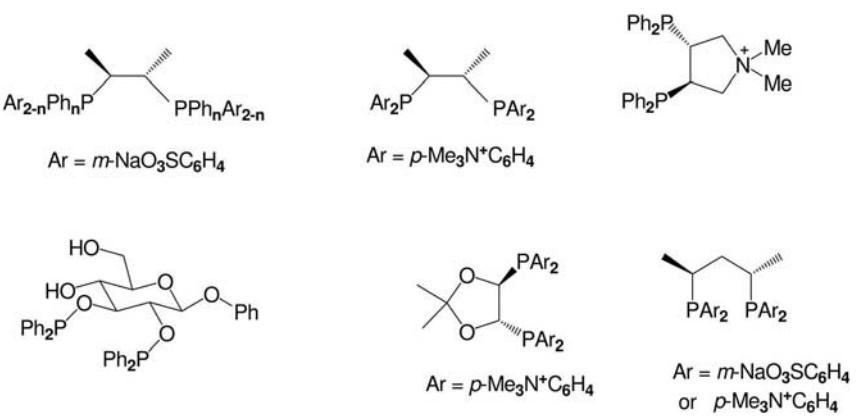


Fig. 7.9 Chiral water soluble ligands.

the selective reduction of the carbonyl group. The coordinative saturation of the latter prevents the coordination of the olefinic double bond but allows the reduction of a carbonyl group by intermolecular hydride transfer [35].

The technique can also be used for the catalytic transfer hydrogenation of carbonyl groups using formate [34, 35] or isopropanol [36] as the hydrogen donor. It is also worth noting, in this context, that Ru/tppts can be used for the selective hydrogenation [37] or transfer hydrogenation [38] of carbohydrates in a monophasic aqueous system (Fig. 7.8).

When chiral water soluble ligands are used the technique can be applied to asymmetric hydrogenations [26]. Some examples are shown in Fig. 7.9.

7.3.3 Carbonylations

The majority of catalytic carbonylations employ palladium catalysts and the water soluble complex, $Pd(tppts)_3$, is easily prepared by reduction of $PdCl_2$/tppts with CO in water at room temperature [39]. Hence, this complex is readily generated *in situ*, under carbonylation conditions. It was shown to catalyze the carbonylation of alcohols [40, 41] and olefins [42–44], in the presence of a Bronsted acid cocatalyst (Fig. 7.10).

The reaction is proposed to involve the formation of an intermediate carbenium ion (hence the need for an acid cocatalyst) which reacts with the Pd(0) complex to afford an alkylpalladium(II) species (see Fig. 7.11) [44].

When a sulfonated diphosphine is used as the ligand, the complex formed with palladium(0) catalyzes the alternating copolymerization of ethylene and

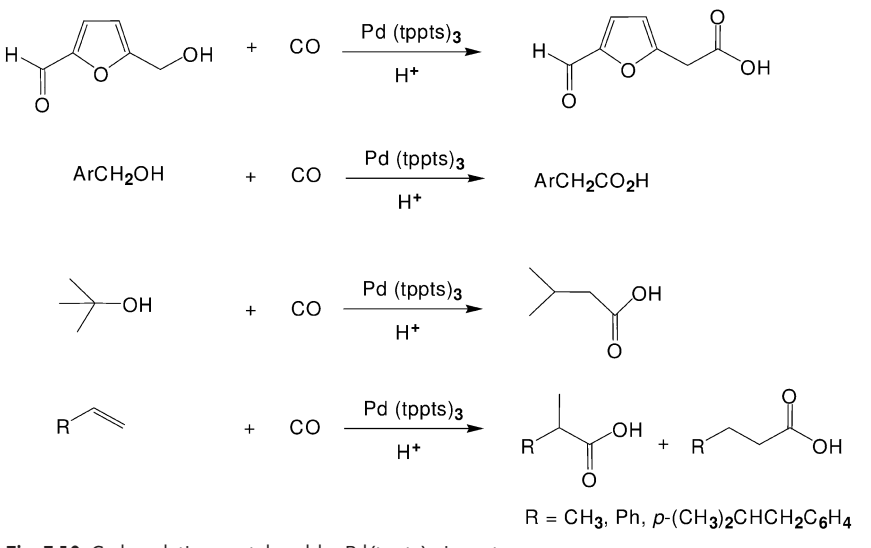


Fig. 7.10 Carbonylations catalyzed by $Pd(tppts)_3$ in water.

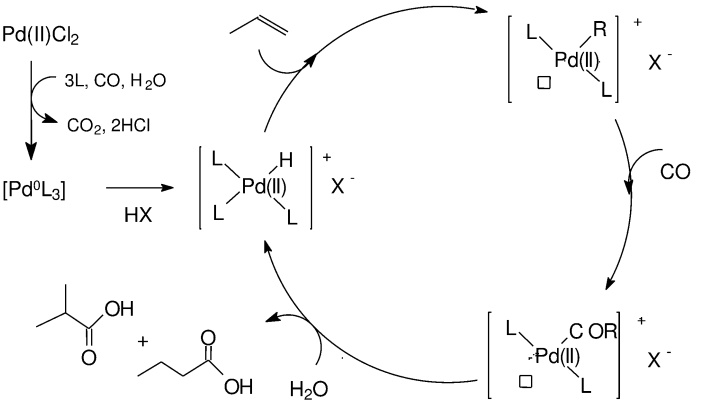
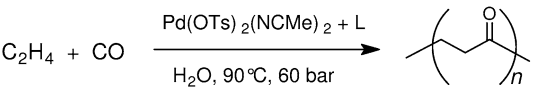
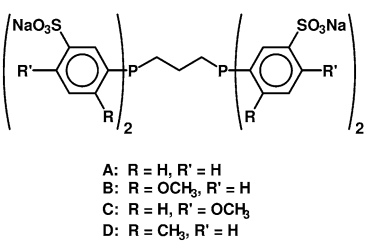


Fig. 7.11 Mechanism of $Pd(tppts)_3$ catalyzed carbonylations.





Ligand	Additive	Activity (kg/g Pd/h)
A	HOTs	4.0
B	HOTs	18.6
B	none	24.4
B	NaOH	0
C	HOTs	1.7
D	HOTs	1.7

Fig. 7.12 Alternating copolymerization of ethylene and CO in water.

CO to give the engineering thermoplastic polyketone, Carilon [45, 46]. Indeed, when a well-defined complex was used (Fig. 7.12), exceptionally high activities were observed [46], with turnover frequencies (TOFs) higher than the conventional catalyst in methanol as solvent.

7.3.4
Other C–C Bond Forming Reactions

Many other C–C bond forming reactions involving organometallic catalysis have been successfully performed in an aqueous biphasic system. Examples are shown in Fig. 7.13 and include Heck [47, 48] and Suzuki couplings [48] and the Rhone-Poulenc process for the synthesis of geranylacetone, a key intermediate in the manufacture of vitamin E, in which the key step is Rh/tppts catalyzed ad-

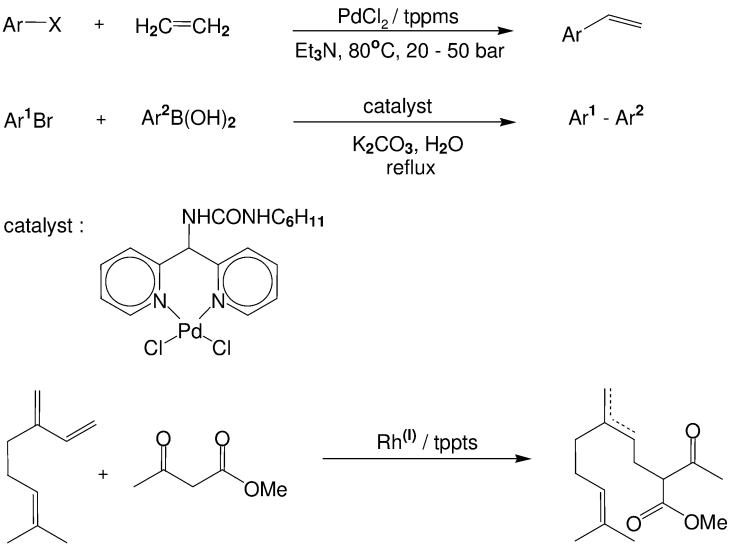


Fig. 7.13 Catalytic C–C bond formation in aqueous biphasic media.

dition of methyl acetoacetate to myrcene [49]. The intermediate beta-keto esters can also be used for the synthesis of pseudo-ionone, a key intermediate in the manufacture of vitamin A. Water soluble variants of the Grubbs Ru catalyst have been used in aqueous biphasic ring opening metathesis polymerization (ROMP) [50].

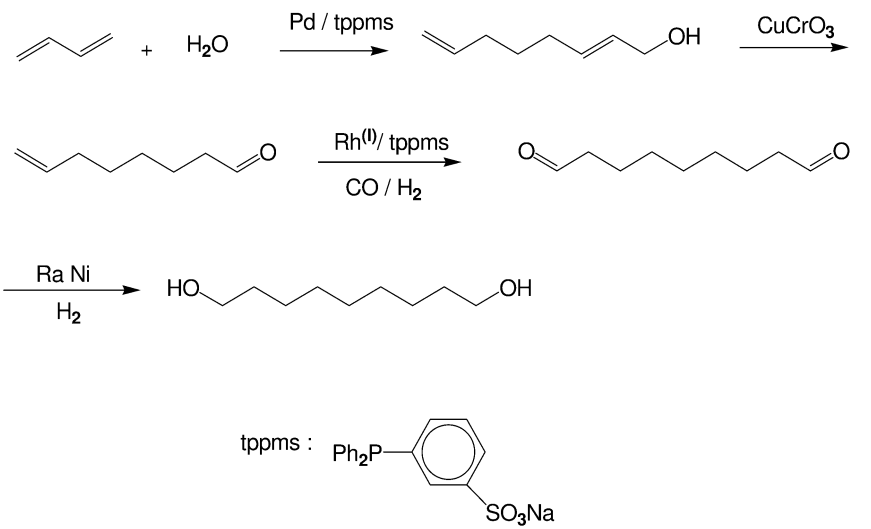


Fig. 7.14 Kuraray process for nonane-1,9-diol.

In the Kuraray process for the production of nonane-1,9-diol two steps involve the use of aqueous biphasic catalysis: Pd/tppms catalyzed telomerization of butadiene with water as a reactant and Rh/tppms catalyzed hydroformylation (Fig. 7.14) [51].

7.3.5
Oxidations

The palladium(II) complex of sulfonated bathophenanthroline was used in a highly effective aqueous biphasic aerobic oxidation of primary and secondary alcohols to the corresponding aldehydes or carboxylic acids and ketones respectively (Fig. 7.15) [52, 53]. No organic solvent was necessary, unless the substrate was a solid, and turnover frequencies of the order of $100\ h^{-1}$ were observed. The catalyst could be recovered and recycled by simple phase separation (the aqueous phase is the bottom layer and can be left in the reactor for the next batch). The method constitutes an excellent example of a green catalytic oxidation with oxygen (air) as the oxidant, no organic solvent and a stable recyclable catalyst.

7.4
Fluorous Biphasic Catalysis

Fluorous biphasic catalysis was pioneered by Horvath and Rabai [54, 55] who coined the term 'fluorous', by analogy with 'aqueous', to describe highly fluorinated alkanes, ethers and tertiary amines. Such fluorous compounds differ markedly from the corresponding hydrocarbon molecules and are, consequently, immiscible with many common organic solvents at ambient temperature although they can become miscible at elevated temperatures. Hence, this provides a basis for performing biphasic catalysis or, alternatively, monophasic catalysis at elevated temperatures with biphasic product/catalyst separation at lower temperatures. A number of fluorous solvents are commercially available (see Fig. 7.16 for example), albeit rather expensive compared with common organic

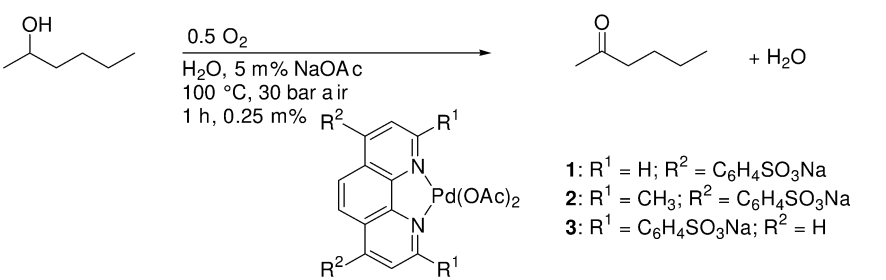


Fig. 7.15 Aqueous biphasic Pd catalyzed aerobic oxidation of alcohols.

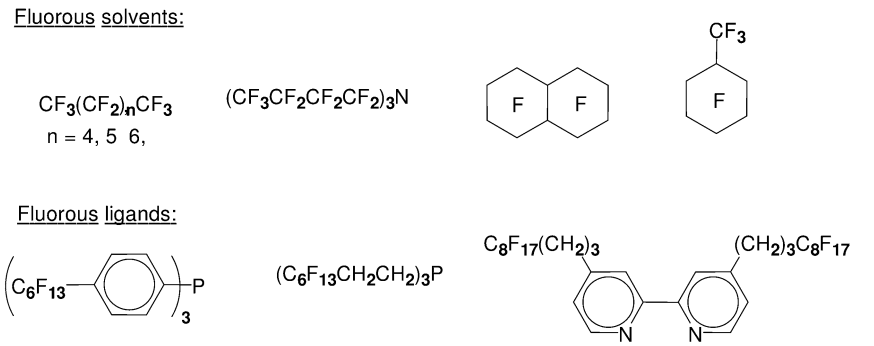


Fig. 7.16 Some examples of fluorous solvents and ligands.

solvents (or water). Barthel-Rosa and Gladysz have published an extensive 'user's guide' to the application of fluorous catalysts and reagents [56].

In order to perform fluorous biphasic catalysis the (organometallic) catalyst needs to be solubilized in the fluorous phase by deploying "fluorophilic" ligands, analogous to the hydrophilic ligands used in aqueous biphasic catalysis. This is accomplished by incorporating so-called "fluorous ponytails".

The attachment of the strongly electron-withdrawing perfluoroalkyl groups would seriously reduce the electron density on, e.g. phosphorus, to an extent that it would no longer be an effective phosphine ligand. This problem is circumvented by placing an "organic" spacer between the perfluoroalkyl moiety and the coordinating atom, generally phosphorus. Some examples of such ligands are shown in Fig. 7.16. It should be noted however that these arguments do not apply to, for example, oxidation reactions, where a strongly electron-withdrawing ligand may actually increase the activity of the catalyst (see later).

7.4.1 Olefin Hydroformylation

As noted earlier, hydroformylation of higher olefins in an aqueous biphasic system is problematic owing to the lack of solubility of the substrate in the aqueous phase. On the other hand, hydroformylation in an organic medium presents the problem of separating the long-chain aldehydes from the catalyst. In contrast, this is not a problem with a fluorous biphasic system where at the elevated reaction temperature the mixture becomes a single phase. Cooling the reaction mixture to room temperature results in a separation into a fluorous phase, containing the catalyst, and an organic phase, containing the aldehyde products. This concept was applied by Horvath and Rabai, in their seminal paper [54], to the hydroformylation of 1-decene in a 1:1 mixture of $C_6F_{11}CF_3$ and toluene. The catalyst was prepared *in situ* from $Rh(CO)_2(acac)$ and $P[CH_2CH_2(CF_2)_5CF_3]_3$ (P/Rh = 40) and the reaction performed at 100 °C and 10 bar CO/H_2. Upon completion of the reaction the reactor was cooled to room

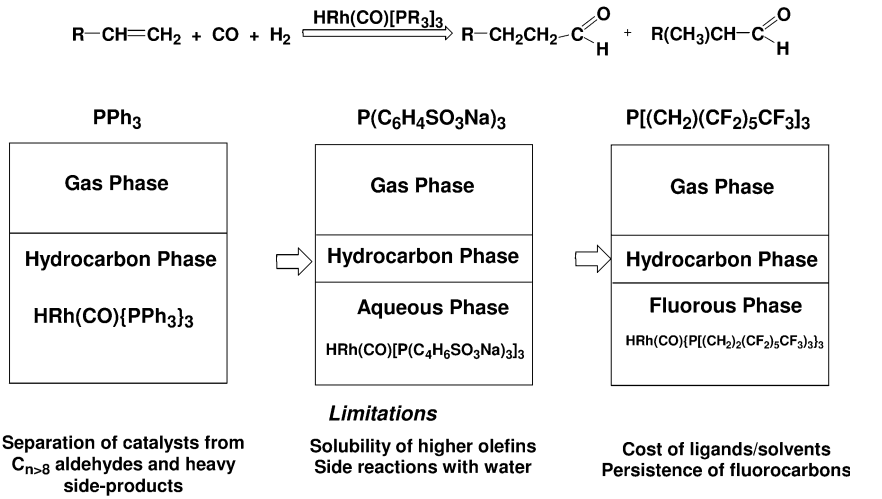


Fig. 7.17 Different concepts for olefin hydroformylation.

temperature when phase separation occurred. When the upper, organic phase was returned to the reactor, with fresh reactants, negligible reaction was observed, demonstrating that catalytically active rhodium species are not leached into the organic phase. It was subsequently shown [57, 58] that recycling of the catalyst phase, in nine consecutive runs, afforded a total turnover number (TON) of more than 35 000. The rhodium losses amounted to 4.2%, which constitutes ca. 1 ppm per mole of aldehyde. Unfortunately there was some leaching of the free ligand into the organic phase, resulting in a slight decrease in (n/i) selectivity (from ca. 92/8 to 89/11), which is dependent on the ligand/Rh ratio. However, the authors noted [57] that this could probably be improved by fine tuning of the solvent and/or ligand. They also emphasized that the system combined the advantages of a monophasic reaction with a biphasic separation. The three different concepts for olefin hydroformylation – organic solvent, aqueous biphasic and fluorous biphasic – are compared in Fig. 7.17.

7.4.2 Other Reactions

The successful demonstration of the fluorous biphasic concept for performing organometallic catalysis sparked extensive interest in the methodology and it has subsequently been applied to a wide variety of catalytic reactions, including hydrogenation [59], Heck and Suzuki couplings [60, 61] and polymerizations [62]. The publication of a special Symposium in print devoted to the subject [63] attests to the broad interest in this area.

Fluorous solvents would seem to be particularly suitable for performing aerobic oxidations, based on the high solubility of oxygen in fluorocarbons, a prop-

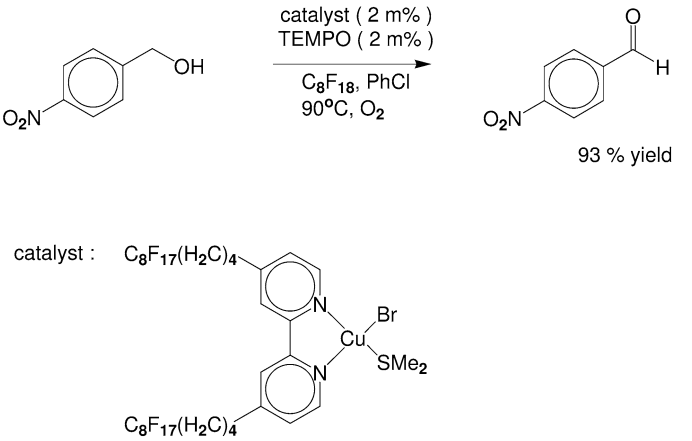


Fig. 7.18 Catalytic oxidation of alcohols in a fluorous medium.

erty which is exploited in their application as blood substitutes [64]. A few examples of catalytic oxidations in fluorous media have been reported [65, 66]. For example, the aerobic oxidation of alcohols was performed in a fluorous medium, using a copper complex of a bipyridine ligand containing perfluorinated ponytails (Fig. 7.18) [66].

Catalytic oxidations with hydrogen peroxide have also been performed in fluorous media (Fig. 7.19) [67].

Notwithstanding the seemingly enormous potential of the fluorous biphasic catalysis concept, as yet a commercial application has not been forthcoming. Presumably the cost of the solvents and ligands is a significant hurdle, and although the catalyst and products are well-partitioned over the two phases there is a finite solubility of the catalyst in the organic phase which has to be coped with. Perhaps an even more serious problem is the extremely long life-

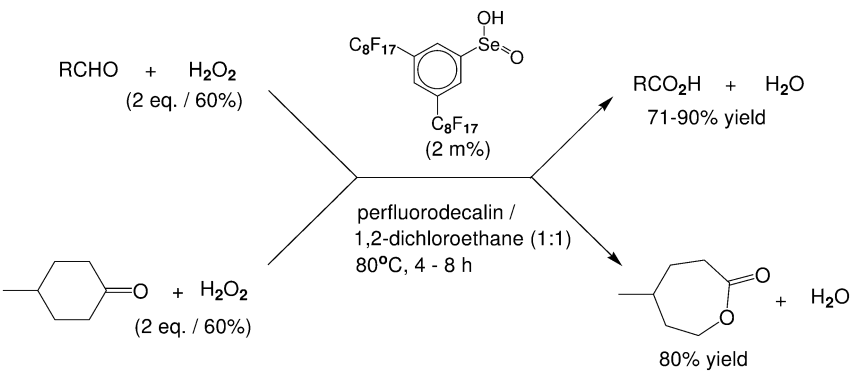


Fig. 7.19 Catalytic oxidations with hydrogen peroxide in fluorous media.

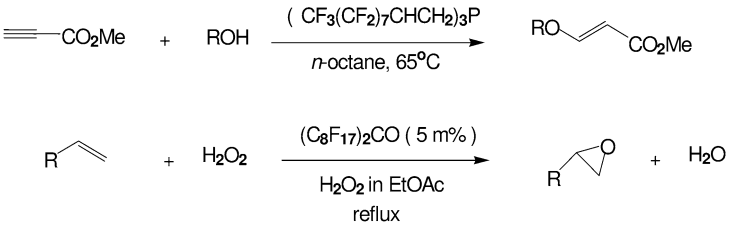


Fig. 7.20 Fluorous catalysis without fluorous solvents.

time of fluorocarbons in the environment which, even though they are chemically inert, essentially nontoxic and are not, in contrast to their cousins the CFCs, ozone-depleting agents, is still a matter for genuine concern.

In this context it is interesting to note the recent reports of *fluorous catalysis without fluorous solvents* [68]. The thermomorphic fluorous phosphines, $P[(CH_2)_m(CF_2)_7CF_3]_3$ ($m=2$ or 3) exhibit ca. 600-fold increase in n-octane solubility between –20 and 80 °C. They catalyze the addition of alcohols to methyl propiolate in a monophasic system at 65 °C and can be recovered by precipitation on cooling (Fig. 7.20) [68]. Similarly, perfluoroheptadecan-9-one catalyzed the epoxidation of olefins with hydrogen peroxide in e.g. ethyl acetate as solvent [69]. The catalyst could be recovered by cooling the reaction mixture, which resulted in its precipitation.

Presumably this technique can be applied to other examples of (organometallic) catalysis. We also note that catalysis can also be performed in supercritical carbon dioxide ($scCO_2$) as solvent (see next section).

7.5 Supercritical Carbon Dioxide

7.5.1 Supercritical Fluids

Supercritical fluids (SCFs) constitute a different category of neoteric solvents to the rest discussed in this chapter since they are not in the liquid state. The critical point (Fig. 7.21) represents the highest temperature and pressure at which a substance can exist as a vapor in equilibrium with a liquid. In a closed system, as the boiling point curve is ascended, with increasing pressure and temperature, the liquid becomes less dense, owing to thermal expansion, and the gas becomes denser as the pressure increases. The densities of the two phases converge and become identical at the critical point, where they merge and become a SCF. Hence the properties, e.g. density, viscosity and diffusivity, of a SCF are intermediate between a liquid and a vapor. SCFs also mix well with gases, making them potentially interesting media for catalytic reactions with hydrogen, carbon monoxide and oxygen.

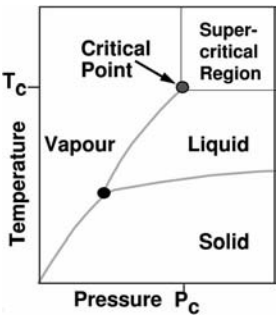


Fig. 7.21 A phase diagram illustrating the supercritical region.

7.5.2 Supercritical Carbon Dioxide

Several features of $scCO_2$ make it an interesting solvent in the context of green chemistry and catalysis. For carbon dioxide the critical pressure and temperature are moderate: 74 bar and 31 °C, respectively. Hence the amount of energy required to generate supercritical carbon dioxide is relatively small.

In addition, carbon dioxide is nontoxic, chemically inert towards many substances, nonflammable, and simple depressurization results in its removal.

It is miscible with, e.g. hydrogen, making it an interesting solvent for hydrogenation and hydroformylation (see below). Although it is a greenhouse gas its use involves no net addition to the atmosphere; it is borrowed as it were. Its main uses are as a replacement for VOCs in extraction processes. For example it is widely used for the decaffeination of coffee where it replaced the use of a chlorinated hydrocarbon. More recently, it has been commercialized as a replacement for trichloroethane in dry cleaning applications [70]. In the last decade attention has also been focused on the use of $scCO_2$ as a solvent for green chemistry and catalysis [71–74]. The pre-existence of an established SCF extraction industry meant that the necessary equipment was already available.

7.5.3 Hydrogenation

The most extensively studied reaction is probably hydrogenation. The miscibility of $scCO_2$ with hydrogen, as noted above, results in high diffusion rates and is a significant advantage. It provides the basis for achieving much higher reaction rates than in conventional solvents. The use of $scCO_2$ as a solvent for catalytic hydrogenations over supported noble metal catalysts was pioneered by Poliakoff [75–77]. For example, the Pd-catalyzed hydrogenation of a variety of functional groups, such as C=C, C=O, C=N and NO_2 was shown [75, 76] to proceed with high selectivities and reaction rates in $scCO_2$. The high reaction rates allowed the use of exceptionally small flow reactors. Chemoselectivities with multifunctional compounds could be adjusted by minor variations in reaction parameters.

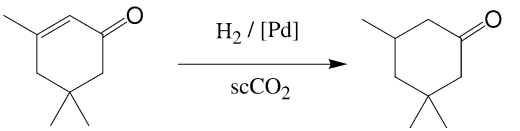
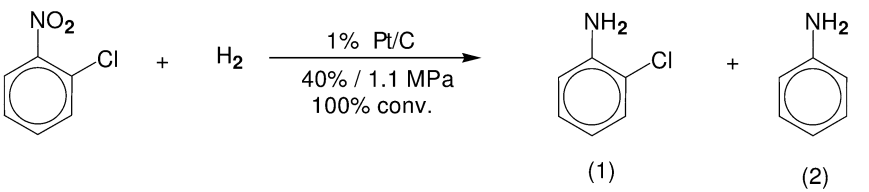


Fig. 7.22 Catalytic hydrogenation of isophorone in $scCO_2$.

The technology has been commercialized, in collaboration with the Thomas Swan company, for the manufacture of trimethyl cyclohexanone by Pd-catalyzed hydrogenation of isophorone (Fig. 7.22) [77].

The multi-purpose plant, which went on stream in 2002, has a production capacity of 1000 tpa (100 kg h^{-1}). The very high purity of the product and high productivities, together with the elimination of organic solvent, are particular benefits of the process. It is a perfect example of the successful transfer of green chemistry from academia to industry. Presumably it will stimulate further industrial application of the technology. Ikariya and coworkers [78], another pioneering group in the area of catalytic hydrogenations in $scCO_2$, recently reported that chemoselective hydrogenation of halogenated nitrobenzene (Fig. 7.23) proceeds very effectively over Pt/C catalysts in $scCO_2$. The rate is significantly enhanced compared to that with the neat substrate and competing dehalogenation is markedly suppressed, affording a higher chemoselectivity. The increased selectivity in $scCO_2$ was tentatively attributed to the generation of small amounts of CO which preferentially cover the more active sites on the platinum surface which are responsible for dehalogenation. Indeed, the addition of small amounts of CO to the neat reaction system caused a marked suppression of competing dehalogenation.

Catalytic asymmetric hydrogenations have also been performed in supercritical carbon dioxide [79–81]. For example, *α*-enamides were hydrogenated in high enantioselectivities comparable to those observed in conventional solvents, using a cationic rhodium complex of the EtDuPHOS ligand (Fig. 7.24) [79]. More recently, catalytic asymmetric hydrogenations have been performed in $scCO_2$ with



	Time (h)	Selectivity (%)	
		1	2
neat	5	95.6	2.1
sc CO_2	2.5	99.7	0.3

Fig. 7.23 Chemoselective hydrogenation of *o*-chloronitrobenzene in $scCO_2$.

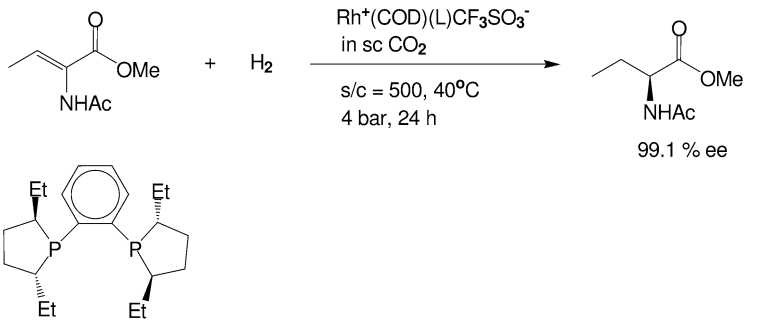


Fig. 7.24 Catalytic asymmetric hydrogenation in $scCO_2$.

Rh complexes containing monodentate perfluoroalkylated chiral ligands [80]. By using a chiral Rh complex immobilized onto alumina, via a phosphotungstic acid linker, asymmetric hydrogenations could be performed in continuous operation using $scCO_2$ as the solvent [81]. Jessop and coworkers [82] compared a variety of neoteric solvents – $scCO_2$, ionic liquids (ILs), ILs with cosolvents and CO_2-expanded ionic liquids (EILs) – in the asymmetric hydrogenation of prochiral unsaturated acids catalyzed by chiral ruthenium complexes. They concluded that the optimum solvent was dependent on the specific substrate used and no one solvent clearly outperforms all others for all substrates. Solvents thought to dissolve significant amounts of hydrogen gave good enantioselectivities for substrates known to be dependent on high H_2 concentrations. Solvents dissolving low amounts of hydrogen, e.g. ionic liquids, were ideal for substrates dependent on minimum H_2 concentrations for high enantioselectivities. Similarly, olefin hydroformylation has been conducted in $scCO_2$ using an immobilized Rh catalyst [83].

7.5.4 Oxidation

Supercritical carbon dioxide is, in principle, an ideal inert solvent for performing catalytic aerobic oxidations as it is nonflammable and completely miscible with oxygen. It is surprising, therefore, that there are so few studies in this area. A recent report describes the aerobic oxidation of alcohols catalyzed by PEG-stabilized palladium nanoparticles in a $scCO_2$/PEG biphasic system [84]. Recently, much interest has also been focused on catalytic oxidations with hydrogen peroxide, generated *in situ* by Pd-catalyzed reaction of hydrogen with oxygen, in $scCO_2$/water mixtures [85]. The system was used effectively for the direct epoxidation of propylene to propylene oxide over a Pd/TS-1 catalyst [86]. These reactions probably involve the intermediate formation of peroxycarbonic acid by reaction of H_2O_2 with CO_2 (Fig. 7.25).

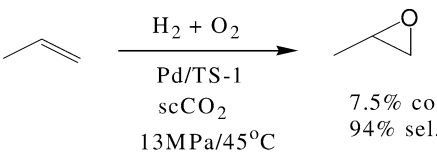


Fig. 7.25 Oxidation of propylene with H_2/O_2 in $scCO_2$.

7.5.5 Biocatalysis

$scCO_2$ is also an interesting solvent for performing bioconversions. The first reports of biocatalysis in $scCO_2$ date back to 1985 [87–89] and in the intervening two decades the subject has been extensively studied [90]. Enzymes are generally more stable in $scCO_2$ than in water and the *Candida antarctica* lipase (Novozym 435)-catalyzed resolution of 1-phenylethanol was successfully performed at temperatures exceeding 100 °C in this solvent [91]. Matsuda et al. found that the enantioselectivity of alcohol acylations catalyzed by Novozyme 435 in $scCO_2$ could be controlled by adjusting the pressure and temperature [92]. The same group recently reported a continuous flow system in $scCO_2$ for the enzymatic resolution of chiral secondary alcohols via Novozyme 435 catalyzed acylation with vinyl acetate (Fig. 7.26) [93]. For example, the kinetic resolution of 1-phenyl ethanol at 9 MPa CO_2 and 40 °C afforded the (*R*)-acetate in 99.8% *ee* and the (*S*)-alcohol in 90.6% *ee* at 48% conversion (E = 1800).

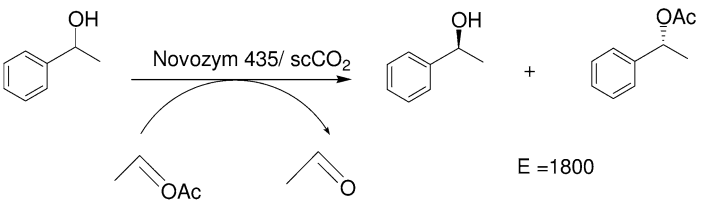


Fig. 7.26 Lipase catalyzed enantioselective transesterification in $scCO_2$.

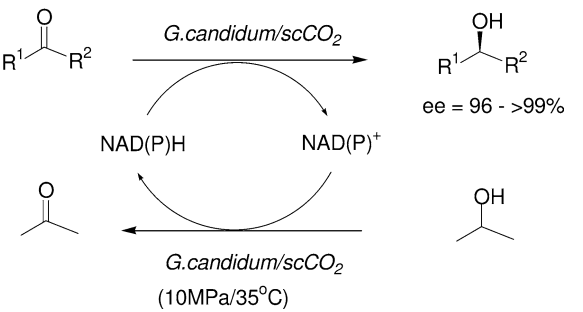


Fig. 7.27 Biocatalytic enantioselective reduction of ketones in $scCO_2$.

Similarly, the enantioselective reduction of prochiral ketones catalyzed by whole cells of *Geotrichum candidum* proceeded smoothly in $scCO_2$ in a semi-continuous flow system (Fig. 7.27) [94].

Enzyme catalyzed oxidations with O_2 have also been successfully performed in $scCO_2$ e.g. using cholesterol oxidase [95] and polyphenol oxidase [88]. The use of $scCO_2$ as a solvent for biotransformations clearly has considerable potential and we expect that it will find more applications in the future.

7.6 Ionic Liquids

Ionic liquids are quite simply liquids that are composed entirely of ions [96, 97]. They are generally salts of organic cations, e.g. tetraalkylammonium, alkylpyridinium, 1,3-dialkylimidazolium, tetraalkylphosphonium (Fig. 7.28). Room temperature ionic liquids exhibit certain properties which make them attractive media for performing green catalytic reactions. They have essentially no vapor pressure and are thermally robust with liquid ranges of e.g. 300 °C, compared to 100 °C for water. Polarity and hydrophilicity/hydrophobicity can be tuned by a suitable combination of cation and anion, which has earned them the accolade, 'designer solvents'.

Ionic liquids have been extensively studied in the last few years as media for organic synthesis and catalysis in particular [98]. For example, the hydroformylation of higher olefins, such as 1-octene, was performed in ionic liquids [99]. Good activities were observed with rhodium in combination with the water-soluble ligand, tppts, described above but the selectivity was low (n/iso ratio = 2.6). In order to achieve both high activities and selectivities special ligands had to be designed (Fig. 7.29). No detectable (less than 0.07%) Rh leaching was observed and the IL phase containing the catalyst could be recycled after separating the product which formed a separate phase. However, the need for rather exotic ligands will presumably translate to higher costs for this process.

As would be expected, high rate accelerations can result when reactions proceeding through ionic intermediates, e.g. carbocations, are performed in ionic liquids. For example, Seddon and coworkers [100] studied the Friedel-Crafts acylation of toluene, chlorobenzene (Fig. 7.30) and anisole with acetyl chloride in [emim][Al_2Cl_7], whereby the ionic liquid is acting both as solvent and catalyst. They ob-

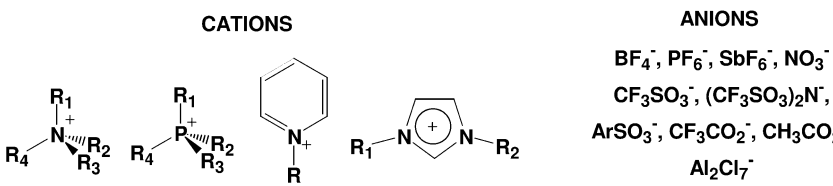
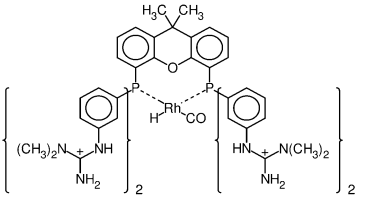
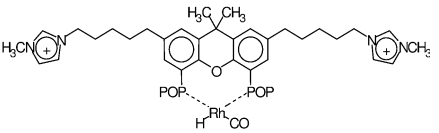


Fig. 7.28 Structures of ionic liquids.



A



B

Catalyst	TOF(h^{-1})	n/iso ratio
$(tppts)_2(CO)RhH$	80	2.6
A	50	20
B	320	49

POP =

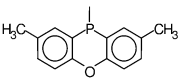
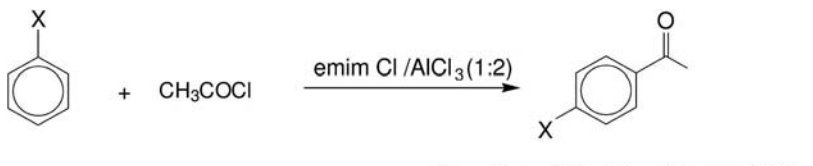
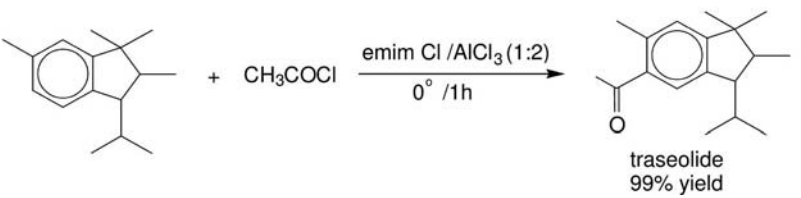


Fig. 7.29 Hydroformylation in ionic liquids.



R	Temp (°C)	Time (h)	Yield (%)
MeO	-10	0.25	99
Me	20	1	98
Cl	20	24	97



traseolide
99% yield

Fig. 7.30 Friedel-Crafts acylation in an ionic liquid.

tained dramatic rate enhancements and improved regioselectivities to the para-isomer compared to the corresponding reactions in molecular organic solvents. Similarly, the fragrance material, traseolide, was obtained in 99% yield as a single isomer (Fig. 7.30). It should be noted, however, that these reactions suffer from the same problems as conventional Friedel-Crafts acylations with regard to work-up and product recovery. The ketone product forms a strong complex with the chloroaluminate ionic liquid, analogous to that formed with aluminum chloride in molecular solvents. This complex is broken by water, leading to aqueous

waste streams containing copious quantities of aluminum salts. More recently, the same group has described the use of chloroindate(III) ionic liquids as catalysts and solvents for Friedel-Crafts acylations [101]. Although indium is a much less active catalyst than aluminum, which limits the substrate scope, product recovery and catalyst recycling are simplified. Metal bistriflamide salts of e.g. Co(II), Mn(II) and Ni(II) were also shown to be effective catalysts (1 mol%) for Friedel-Crafts acylations, either neat or in ionic liquids as solvents [102].

Generally speaking, recycling of catalysts in ionic liquid systems is facilitated when they are ionic compounds (salts) as these are generally very soluble in the ionic liquid phase but insoluble in organic solvents, thus enabling extraction of the product and recycling of the catalyst in the ionic liquid phase. For example, olefin metathesis has been performed in an ionic liquid using a cationic ruthenium allenylidene complex as the catalyst (Fig. 7.31) [103]. It was demonstrated that the catalyst could be recycled in the IL phase but a markedly lower conversion was observed in the third run owing to decomposition of the catalyst, which is also observed in conventional organic solvents. The results were markedly dependent on the anion of the ionic liquid, the best results being obtained in [bmim] [CF_3CO_2].

In the last few years increasing attention has been devoted to conducting biocatalytic transformations in ionic liquids [104–107]. The first report of enzyme-(lipase-) catalyzed reactions in water-free ionic liquids dates from 2000 and involved transesterification, ammoniolysis and perhydrolysis reactions catalyzed by *Candida antarctica* lipase B (Fig. 7.32) [108].

The use of ionic liquids as reaction media for biotransformations has several potential benefits compared to conventional organic solvents, e.g., higher operational stabilities [109] and enantioselectivities [110] and activities are generally at least as high as those observed in organic solvents. They are particularly attractive for performing bioconversions with substrates which are very sparingly soluble in conventional organic solvents, e.g., carbohydrates [111] and nucleosides.

Notwithstanding the numerous advantages of ionic liquids as reaction media for catalytic processes widespread industrial application has not yet been forthcoming. The reasons for this are probably related to their relatively high prices and the paucity of data with regard to their toxicity and biodegradability. The replacement of conventional VOCs with ionic liquids is an obvious improvement with regard to atmospheric emissions but small amounts of ionic liquids will

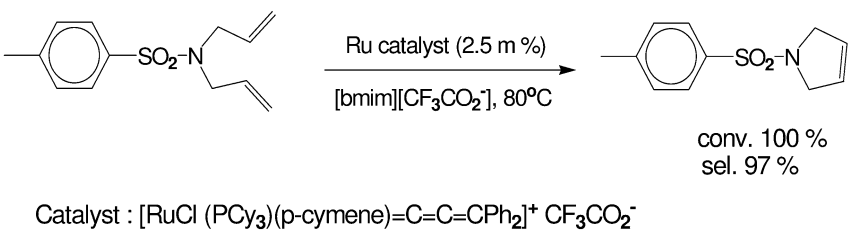


Fig. 7.31 Olefin metathesis in an ionic liquid.

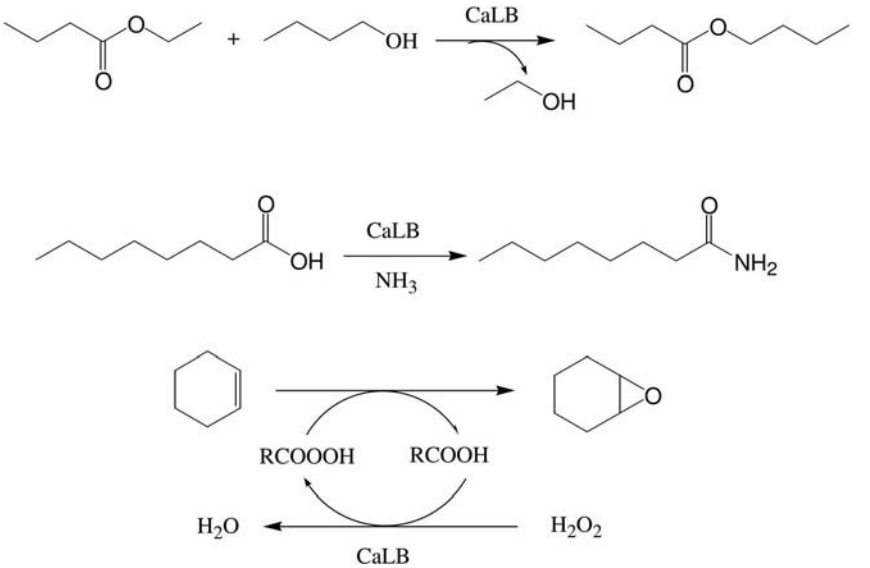
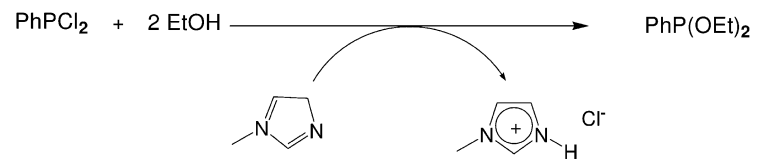
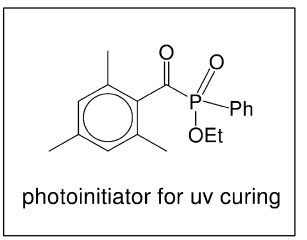


Fig. 7.32 Biotransformations in ionic liquids.

inevitably end up in the environment, e.g., in ground water. Consequently, it is important to establish their effect on the environment, e.g. with regard to their toxicity [112] and biodegradability [113]. Indeed, the current trend in ionic liquid research is towards the development of inexpensive, non-toxic, biodegradable ionic liquids, e.g. based on renewable raw materials [114].

One interesting application of an ionic liquid does not involve its use as a solvent. The company BASF used *N*-methylimidazole to scavenge the HCl formed in the reaction of dichlorophenyl phosphine with ethanol (Fig. 7.33). This results in the formation of *N*-methylimidazoliumchloride (Hmim-Cl), which has a





Process	New	Old
STY (kg/m²/h)	690,000	8

Fig. 7.33 The BASIL process.

melting point of 75 °C and is, hence, an ionic liquid under the reaction conditions. During the process, which has the acronym BASIL (biphasic scavenging utilizing ionic liquids), the ionic liquid separates as a second phase and is readily separated from the product and recycled [115]. The product is a precursor of UV photoinitiators which find a variety of applications. In the conventional process a solid amine hydrochloride is formed from e.g. triethylamine as the acid scavenger. This necessitates the use of large quantities of organic solvents and more complicated work-up. The methylimidazole also acts as a nucleophilic catalyst, resulting in a forty-fold rate enhancement. The new process is also performed in a continuous operation mode and the space time yield was increased from 8 kg m^{-2} h^{-1} to 690 000 kg m^{-2} h^{-1}.

7.7 Biphasic Systems with Supercritical Carbon Dioxide

One problem associated with the use of ILs is recovery of the product and recycling of the catalyst. If this is achieved by extraction with a volatile organic solvent then it is questionable what the overall gain is. An attractive alternative is to use $scCO_2$ as the second phase, whereby the catalyst remains in the IL phase and the product is extracted into the $scCO_2$ phase. This concept has been successfully applied to both homogeneous metal catalysis [116] and biocatalytic conversions [117]. We have recently applied the concept of using a 'miscibility switch' for performing catalytic reactions in IL/$scCO_2$ mixtures [118]. This takes advantage of the fact that, depending on the temperature and pressure, $scCO_2$ and IL mixtures can be mono- or biphasic. Hence, the reaction can be performed in a homogeneous phase and, following adjustment of the temperature and/or pressure, the product separated in the $scCO_2$ layer of the biphasic system.

Other combinations with $scCO_2$ have also been considered which dispense with the need for an ionic liquid altogether. For example, a biphasic water/$scCO_2$ system, whereby the catalyst, e.g. a metal complex of tppts, resides in the water phase and the product is removed in the $scCO_2$ phase [119, 120]. The system has its limitations: the catalyst needs to be water soluble and all reaction components must be stable towards the acidic conditions (pH 3) of carbonic acid. It is not effective for reactions in which the catalyst, substrate or reagent is insoluble in water or sensitive to the low pH of the aqueous phase [121]. More recently, an attractive system comprising a biphasic mixture of poly(ethylene glycol) (PEG) to dissolve the catalyst and $scCO_2$ as the extractive phase was used for the $RhCl(Ph_3P)_3$-catalyzed hydrogenation of styrene [122]. PEGs have the advantage over ILs that they are much less expensive and are nontoxic (analogous to CO_2, they are approved for use in foods and beverages). They are, moreover, miscible with common organic ligands and, in the above example, the catalyst was stable and recyclable in the PEG phase. Similarly, biocatalytic transformations have also been performed in a PEG/$scCO_2$ biphasic system (Fig. 7.34) [123].

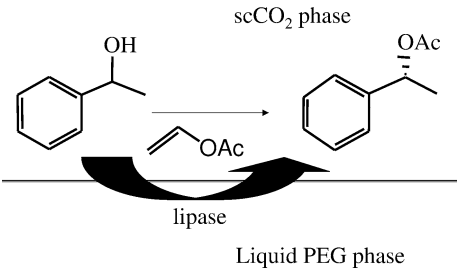


Fig. 7.34 Lipase-catalyzed transesterification in PEG/$scCO_2$.

7.8
Thermoregulated Biphasic Catalysis

Another approach to facilitating catalyst separation while maintaining the benefits of homogeneous catalysis involves the use of thermoregulated biphasic catalysis [124, 125], whereby the catalyst is dissolved in a particular solvent at one temperature and insoluble at another. For example, a diphosphine ligand attached to an ethylene oxide/propylene oxide block copolymer (Fig. 7.35) afforded rhodium complexes that are soluble in water at room temperature but precipitate on warming to 40 °C. The driving force for this inverted temperature dependence on solubility is dehydration of the ligand on heating. Hence, a rhodium-catalyzed reaction such as hydrogenation or hydroformylation can be performed at room temperature in a single phase and the catalyst separated by precipitation at a higher temperature. An added advantage is that runaway conditions are never achieved since the catalyst precipitates and the reaction stops on raising the temperature. This principle has also been applied to biotransformations by attaching enzymes to EO/PO block copolymers [126].

7.9
Conclusions and Prospects

The employment of catalytic methodologies – homogeneous, heterogeneous and enzymatic – in water or supercritical carbon dioxide as the reaction medium holds much promise for the development of a sustainable chemical manufacturing industry. Water is cheap, abundantly available, non-toxic and non-inflammable and the use of aqueous biphasic catalysis provides an ideal basis for recovery

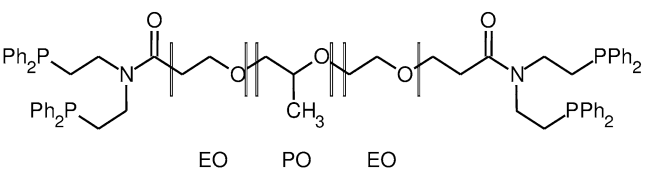


Fig. 7.35 Ethylene oxide/propylene oxide block copolymer.

and recycling of the (water-soluble) catalyst. Water is also the ideal solvent for many processes catalysed by Nature's catalysts, enzymes. Hence, the use of water as a reaction medium meshes well with the current trend towards a sustainable chemical industry based on the utilization of renewable raw materials rather than fossil fuels as the basic feedstock.

Supercritical carbon dioxide has many potential benefits in the context of sustainability. In common with water, it is cheap, abundantly available, non-toxic and non-inflammable. It is also an eminently suitable solvent for homogeneous, heterogeneous and biocatalytic processes and is readily separated from the catalyst and products by simple release of pressure. Reaction rates are very high in $scCO_2$, owing to its properties being intermediate between those of a gas and a liquid. Biphasic systems involving $scCO_2$ with, for example, an ionic liquid or polyethylene glycol also hold promise as reaction media for a variety of catalytic processes integrated with product separation and catalyst recycling.

The ultimate in sustainable catalytic processes is the integration of chemocatalytic and/or biocatalytic steps into catalytic cascade processes that emulate the metabolic pathways of the cell factory. It is an esthetically pleasing thought that, in the future, fuels, chemicals and polymers could be obtained from carbon dioxide and water as the basic raw materials via biomass, using sunlight as the external source of energy and water and supercritical carbon dioxide as solvents. The important difference between this bio-based scenario and the current oil-based one is the time required for renewal of the feedstocks.

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8
Chemicals from Renewable Raw Materials

8.1
Introduction

For the last 70 years or so the chemical industry has been based on crude oil (petroleum) and natural gas as basic raw materials, hence the name petrochemicals. This may not be so for much longer, however. The chemical industry is currently on the brink of a new revolution, based on the switch from fossil resources to renewable agriculture-based raw materials. From a distance the production facility of Cargill in Blair, Nebraska looks very much like a small oil refinery or medium-sized petrochemicals plant. However, closer inspection reveals that it is a corn-processing plant; a biorefinery producing, *inter alia*, high-fructose corn syrup, ethanol and lactic acid. As James R. Stoppert, a senior executive of Cargill pointed out, the chemical industry is based on carbon and it does not matter if the carbon was fixed 2 million years ago or 6 months ago [1].

The necessity to switch from nonrenewable fossil resources to renewable raw materials, such as carbohydrates and triglycerides derived from biomass, was an important conclusion of the Report of the Club of Rome in 1972 [2]. It should be noted, however, that ca. 80% of the global production of oil is converted to thermal or electrical energy. If the world is facing an oil crisis it is, therefore, an energy crisis rather than a raw materials crisis for the chemical industry. Indeed, there are sufficient reserves of fossil feedstocks to satisfy the needs of the chemical industry for a long time to come.

Nonetheless, a (partial) switch to 'renewables' is desirable for other reasons, such as biocompatibility, biodegradability and lower toxicity, i.e. renewable raw materials leave a smaller 'environmental footprint' [3]. That the chemical industry has been slow to make the transition, in the three decades following the Report of the Club of Rome, is a consequence of the fact that oil and natural gas are excellent basic feedstocks and highly atom efficient, low waste, catalytic procedures are available for their conversion into commodity chemicals. The same cannot be said for the fine chemicals industry where processes are, generally speaking, much less efficient in many respects and there is considerable room for improvement.

Products based on renewable raw materials are derived from CO_2 and H_2O via photosynthesis and, following their use, are ultimately returned to the bio-

sphere as CO_2 and H_2O via biodegradation. In principle, they are CO_2 neutral and, hence, have a beneficial effect on greenhouse emissions. Furthermore, in an era of steeply rising oil and natural gas prices, they are becoming more dependable and relatively less expensive.

Renewable raw materials can contribute to the sustainability of chemical products in two ways: (i) by developing greener, biomass-derived products which replace existing oil-based products, e.g. a biodegradable plastic, and (ii) greener processes for the manufacture of existing chemicals from biomass instead of from fossil feedstocks. These conversion processes should, of course, be catalytic in order to maximize atom efficiencies and minimize waste (E factors) but they could be chemo- or biocatalytic, e.g. fermentation [3–5]. Even the chemocatalysts themselves can be derived from biomass, e.g. expanded corn starches modified with surface SO_3H or amine moieties can be used as recyclable solid acid or base catalysts, respectively [6].

What are renewable raw materials and how do their prices compare with oil, coal and natural gas? It has been estimated [7] that the global biomass production amounts to ca. 10^{11} tons per annum, only 3% of which is cultivated, harvested and used (food and non-food). It consists of 75% carbohydrates and 20% lignin, with the remaining 5% comprising oils, fats, proteins and terpenes. In a traditional agricultural economy farmers produce grains for food and feed. In a biobased economy farmers still produce these but the non-food parts of plants, e.g. wheat straw, corn stover and sugar cane bagasse, are used as raw materials for fuels and commodity chemicals.

The prices of some pertinent examples of agriculture-based raw materials (biomass) are compared with oil and coal in Table 8.1. It is obvious that the cheapest source of carbon is agricultural waste, i.e. waste plant biomass such as corn stover, wheat straw and sugar cane bagasse, which consists primarily of lignocellulose.

It has been estimated [3] that enough waste plant biomass is generated in the United States to produce all of the organic chemicals currently manufactured by the US chemical industry and supply a significant fraction of its liquid transportation fuel needs. For example, one ton of wheat straw affords ca. 600 kg of carbohydrates and ca. 200 kg of lignin. The former can be converted, by fermen-

Table 8.1 Prices of various raw materials.

Raw material	Average world market price (€/kg)
Crude oil	0.175 (0.400)
Coal	0.035
Corn	0.080
Wheat straw	0.020
Sugar	0.180
Ethanol	0.400
Ethylene	0.400

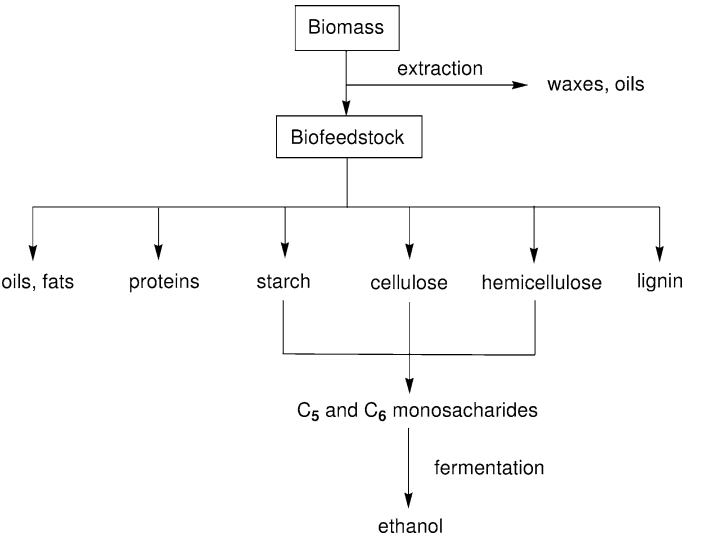


Fig. 8.1 The biorefinery.

tation, to 85 gallons (ca. 180 kg) of ethanol and the latter burned to generate electricity.

The transition to a biobased economy is currently in an intermediate phase where certain commodity chemicals, e.g. lactic acid, are being produced from corn starch. Ultimately, however, economically viable production of bulk chemicals and liquid fuels will only be possible from inexpensive lignocellulose, generated intentionally by cultivation of forage crops, e.g. hay, or derived from waste crop and forestry residues.

One can envisage the future production of liquid fuels and commodity chemicals in a 'biorefinery'. Biomass is first subjected to extraction to remove waxes and essential oils. Various options are possible for conversion of the remaining 'biofeedstock', which consists primarily of lignocellulose. It can be converted to synthesis gas ($CO + H_2$) by gasification, for example, and subsequently to methanol. Alternatively, it can be subjected to hydrothermal upgrading (HTU), affording liquid biofuels from which known transport fuels and bulk chemicals can be produced. An appealing option is bioconversion to ethanol by fermentation. The ethanol can be used directly as a liquid fuel and/or converted to ethylene as a base chemical. Such a 'biorefinery' is depicted in Fig. 8.1.

Recent advances in molecular genetics and metabolic engineering have laid the foundations for the transition from an oil-based to a biobased economy. The one remaining bottleneck, where technological breakthroughs are still needed, is the efficient conversion of lignocellulose to lignin and (hemi)cellulose [3].

8.2 Carbohydrates

According to an authorative estimate, ca. 10^{11} tons per annum of carbohydrates, mainly in the form of lignocellulose, grow and decay every year. The annual wood harvest is small by comparison and has been estimated at ca. 2×10^9 tons per annum. Approximately 5% of the wood harvest is converted into pulp and a minor fraction (5×10^6 tons per annum) of the latter into cellulose. Approximately 60% of this is processed into fibers and films or converted into cellulose esters and ethers [8]. Hence, there would be sufficient lignocellulose available to satisfy all of the raw materials needs many times over. It is, moreover, the cheapest source of biomass-derived energy [9] and a considerable research effort, dating back to the first energy crisis, has been devoted to the possible production of ethanol from lignocellulose [10, 11].

The native structure of lignocellulose, which is very complex, renders it resistant to enzymatic hydrolysis. The major component is cellulose, a $\beta1\rightarrow4$ polymer of glucose (see Fig. 8.2a), which accounts for 35–50% of the mass. Lignocellulose also contains 20–35% hemicellulose, a complex polymer of pentoses and hexoses and 10–25% lignin [10, 12]. Techniques to convert lignocellulose into a fermentable sugar mixture have been under development for over 20 years and are now entering use.

Digestion of lignocellulose, to render it susceptible to hydrolysis, can be performed in a number of ways [10, 13, 14] but few of these satisfy the green commandments. There seems to be a trend towards aqueous pretreatment, such as the steam explosion that is used by Iogen (Canada) [15, 16], followed by enzy-

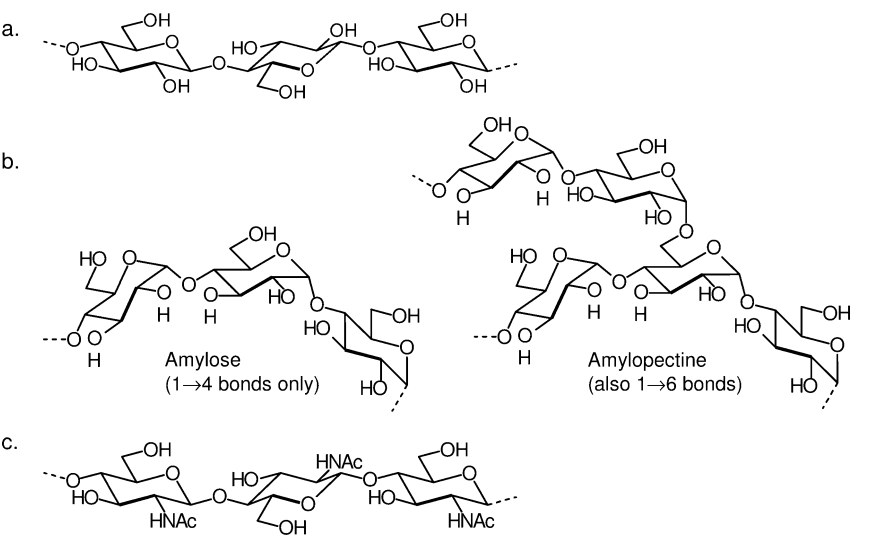


Fig. 8.2 Carbohydrate structures: (a) cellulose, (b) amylose and amylopectin, (c) chitin.

matic hydrolysis in the presence of cellulases. Due to the presence of hemicellulose, the resulting sugar mixture contains pentoses, which resist fermentation by *Saccharomyces* species (see later).

Starch is the other carbohydrate-based feedstock. Approximately 10 Mt is produced annually from corn (maize), wheat and potato, out of a total agricultural production of 1.6 Gt a^{-1} carbohydrate equivalents. A minor fraction of starch is amylose, a linear $a1 \rightarrow 4$ polymer of glucose (Fig. 8.2 b). The native structure of amylose is helical; loose random coils are formed upon dissolution in water. The branched glucose polymer amylopectin is the major (approximately 75%) component of starch.

The industrial processing of starch into glucose, which provides the cheapest access to the latter, is very well developed industrially [17]. Acidic as well as enzymatic hydrolysis is employed. The development of enzymes that maintain their activity under the preferred process conditions is a major activity of the large suppliers of industrial enzymes.

A minor source of carbohydrates that should be mentioned, nevertheless, is chitin, a polymer of *N*-acetyl-D-glucosamine (see Fig. 8.2 c) that resembles cellulose in its structure and general behavior. Chitin is extracted at a 150 kt a^{-1} scale from shellfish waste [18], which is a tiny fraction of the amount that grows and decays every year.

Non-food applications of poly- and oligosaccharides and glucose and their derivatives are many, but few of these involve the use of such carbohydrates as a chemical. The reason is obvious: the complex structure of carbohydrates and the large number of reactive groups make carbohydrate-based organic chemistry highly complicated. Nature has developed its own (also highly complex) ways to deal efficiently with structural complexity. Hence, it is not surprising that nearly all examples of manufacture of chemicals from carbohydrates build on Nature's achievements, using fermentation.

8.2.1 Chemicals from Glucose via Fermentation

Fermentation is the reproduction of microorganisms in the presence of a source of carbon and energy (such as sugar) and various nutrients. The products of fermentation fall into five major categories:

1. More microbial cells
2. Macromolecular products excreted by the cells, such as enzymes and polysaccharides
3. Primary metabolites, such as acetic acid, acetone, amino acids, citric acid, vitamins, that are essential for cellular growth or result from the conversion of glucose into energy for the living cell
4. Secondary metabolites, such as antibiotics, dyes, pigments, aroma compounds, poisons, that are not essential for cellular growth but serve the survival of the species

5. Microbial transformations of foreign substrates, often referred to as precursor fermentation. It may be preferred to perform such transformations with whole cells rather than isolated enzymes when the latter approach would involve the recycling of expensive cofactors. Hence, precursor fermentations are often preferred for conducting redox transformations (see Chapter 6).

Some primary metabolites have been produced via anaerobic fermentation in the past, before the onset of the petrochemical age. Thus, acetone was produced via acetone–butanol fermentation in *Clostridium acetobutylicum* from 1914 onwards. Some chemicals are produced via fermentation without any competition from chemical procedures, owing to the efficiency of the former (citric acid, L-glutamic acid, L-proline) or because the complexity of the product renders chemical synthesis unattractive (β-lactam antibiotics, vitamin B_{12}). With the present-day fermentation technology it seems a fair estimate that a fermentative process, to compete with a chemical one, should replace at least four to five chemical steps of moderate complexity. A much wider adoption of the biotechnological production of chemicals is hampered by two factors that put it at a competitive disadvantage compared with chemical processes: a low space-time yield and high operating costs.

Space-time yield (STY) sets the capital costs of the production facility and an STY of 100 g L^{-1} d^{-1} has often been mentioned as the minimum for the profitable production of a building block of intermediate complexity. Fermentation processes are often much less productive, due to regulation (inhibition) and toxicity problems.

Regulation is a blanket concept that covers all negative (i.e. stabilizing) feedback mechanisms of a product on its biosynthesis, such as inhibition of an enzyme by one of the downstream products or transcriptional control, which acts at the level of the gene.

Strain improvement, to increase the stoichiometric yield, the product concentration and the STY, was, until the early 1990s, generally achieved via classical techniques, i.e. by putting the species under selective pressure. Because the progress depends on the natural mutation frequency, the latter is routinely augmented by the application of mutagenic chemicals, UV radiation etc.

Classical strain improvement can be used, for example, to address the regulation of an amino acid by selecting mutants that are able to grow in the presence of a mimic that has similar regulation characteristics. The classical approach involves random mutagenesis and addresses the whole genome. Hence, the phenotype is primarily targeted and the actual mechanism is not easily subjected to rational control.

A recently introduced technique that also targets the phenotype is whole genome shuffling. This latter approach involves the amplification of the genetic diversity within a population through genetic recombination [19, 20]. Next, similar to the traditional procedure, the newly created library is screened for mutants with the desired properties. Its main advantage is that improvement is much faster than is possible with classical mutation and selection as described above.

Metabolic pathway engineering, in contrast, is a rational approach that targets selected enzymes or genes [21, 22]. It has eclipsed the classical approach in the course of the 1990s due to its much better rate of improvement. Metabolic pathway engineering involves the reprogramming of the metabolic network, based on recombinant DNA technology and knowledge of whole genomes. The objective is to optimize the flow of carbon into the biosynthesis of the desired product. Commonly followed basic approaches are:

1. The removal of kinetic bottlenecks by removing regulation and/or by amplifying the genes that code for the rate-limiting enzymes. Alternatively, the WT enzymes may be replaced by mutated or heterologous ones with a different control architecture.
2. Directing metabolic flow from a common biosynthetical intermediate to the desired product by overexpressing the branchpoint enzyme.
3. Reprogramming the central metabolism, if necessary, to supply the required redox equivalents and metabolic energy, usually in the form of ATP.
4. Fine-tuning of the enzymes in the biosynthetic pathway to prevent the accumulation of intermediates.

In the cases described below we will generally focus on metabolic pathway engineering rather than on classical strain improvement, for obvious reasons. One should be aware, however, that the classical approach has strengths that can make it a powerful partner of the rational approach. Thus, regulation problems have been addressed by the development of a feedback-resistant enzyme, using selective pressure and random mutagenesis as described above, in a research species, followed by introduction of the altered gene in the production species via recombinant techniques.

High operating costs put microbial synthesis of chemicals at a competitive disadvantage that is mainly due to the downstream processing (DSP), which is inherently more complex than the work-up of a chemical transformation. In the DSP of a microbial transformation, biomass is separated and the – often dilute – product is isolated from the aqueous supernatant and subsequently purified to the desired grade. The necessary precipitation, extraction and/or distillation steps may consume organic solvents, generate salts and consume energy on a scale that defeats the green purpose of a fermentative process.

In the examples presented below fermentation is compared with traditional chemical manufacturing processes, emphasizing the strengths and weaknesses of fermentative production techniques from a Green Chemistry viewpoint.

8.2.2 Ethanol

The anaerobic fermentation of ethanol from sugar (Fig. 8.3) goes back to the Stone Age. In 1997, the fermentation of ethanol, mainly from sugar cane, molasses (Brazil) and corn (USA), amounted to 24 Mt worldwide, dwarfing the chemical production of 2.6 Mt a^{-1} [23]. Iogen (Canada) produces ethanol from

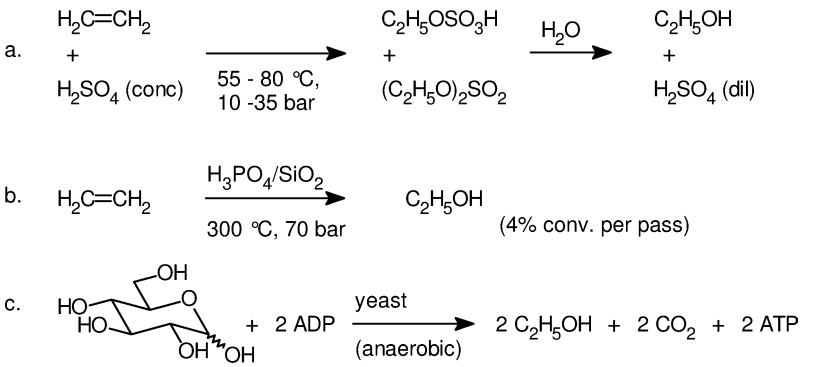


Fig. 8.3 Production of ethanol: (a), (b) chemically, from ethene; (c) via anaerobic fermentation.

straw at pilot-scale (approx. 800 t a^{-1}); a 130 kt a^{-1} plant is expected to be completed in 2007 [15, 16].

The chemical production of ethanol involves acid-catalyzed hydration of ethene using either sulfuric acid (Fig. 8.3 a) [23] or a solid catalyst, such as H_3PO_4/SiO_2, in a recycle process (Fig. 8.3 b). A major disadvantage of these processes is the low conversion per pass of 4% [23], which is a consequence of the short contact time that is maintained to limit the formation of diethyl ether and ethene oligomers.

Under anaerobic conditions, many aerobic microbes switch their metabolism and excrete partially oxidized intermediates, such as ethanol or lactic acid, to maintain redox balance. The pathway involved, the glycolysis pathway, plays a leading role in all fermentations and is outlined in Fig. 8.4.

Glucose (Glc) is taken up and phosphorylated into glucose-6-phosphate (Glc6P), with consumption of ATP. Isomerization and phosphorylation afford fructose-1,6-bisphosphate (Fru1,6P_2), which is cleaved into two triose molecules: D-glyceraldehyde-3-phosphate (GA3P) and dihydroxyacetone monophosphate (DHAP). These are equilibrated by triose phosphate isomerase as only GA3P is metabolized further, except approximately 5 mol% of DHAP that leaks out of the pathway via reduction to glycerol, which is excreted as a side-product.

GA3P is subsequently converted, in a number of steps, into pyruvate (PYR), which is the branch-point between fermentation and respiration. *Saccharomyces* species are particularly well adapted to the anaerobic production of ethanol, via decarboxylation and reduction of PYR, to the near-exclusion of other metabolites. On account of this latter characteristic, as well as its high ethanol tolerance, *Saccharomyces* is the preferred organism to produce ethanol from hexoses.

The pentoses, such as xylose (Xyl), that result from the hydrolysis of lignocellulose (see above) resist fermentation by *Saccharomyces*, because it lacks an efficient mechanism to convert Xyl into xylulose (Xlu). The isomerization redox interconversion pathway of Xyl and Xlu, via xylitol, that is native to *Saccharomyces*, is inefficient due to a cofactor incompatibility (see Fig. 8.5) and results in a redox imbalance and the accumulation of xylitol [24]. Many bacteria, in contrast,

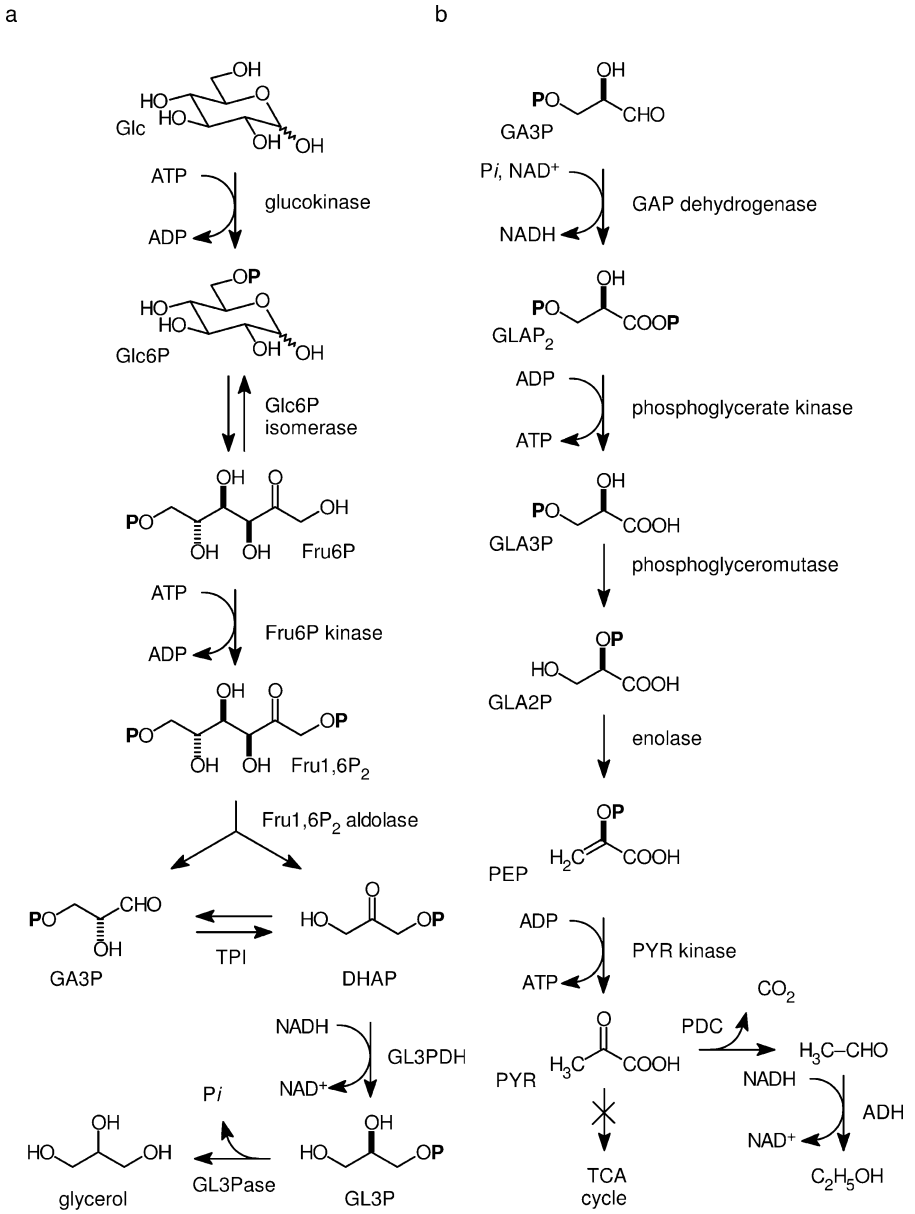


Fig. 8.4 The glycolysis pathway in *Saccharomyces* and the biosynthesis of ethanol and glycerol. Compounds: DHAP, dihydroxylacetone monophosphate; Fru6P, D-fructose-6-phosphate; Fru1,6P_2, D-fructose-1,6-bisphosphate; GA3P, D-glyceraldehyde-3-phosphate; GL3P, *sn*-glycerol-3-phosphate; GLAP$_2$, phosphoglycerate-3-phosphate; GLA3P, glycerate-3-phosphate; GLA2P, glycerate-2-phosphate; Glc, D-glucose; Glc6P, D-glucose-6-phosphate; PEP, phosphoenolpyruvate; PYR, pyruvate. Enzymatic activities: GL3Pase, glycerol-3-phosphatase; ADH, alcohol dehydrogenase; PDC, pyruvate decarboxylase; TPI: triose phosphate isomerase.

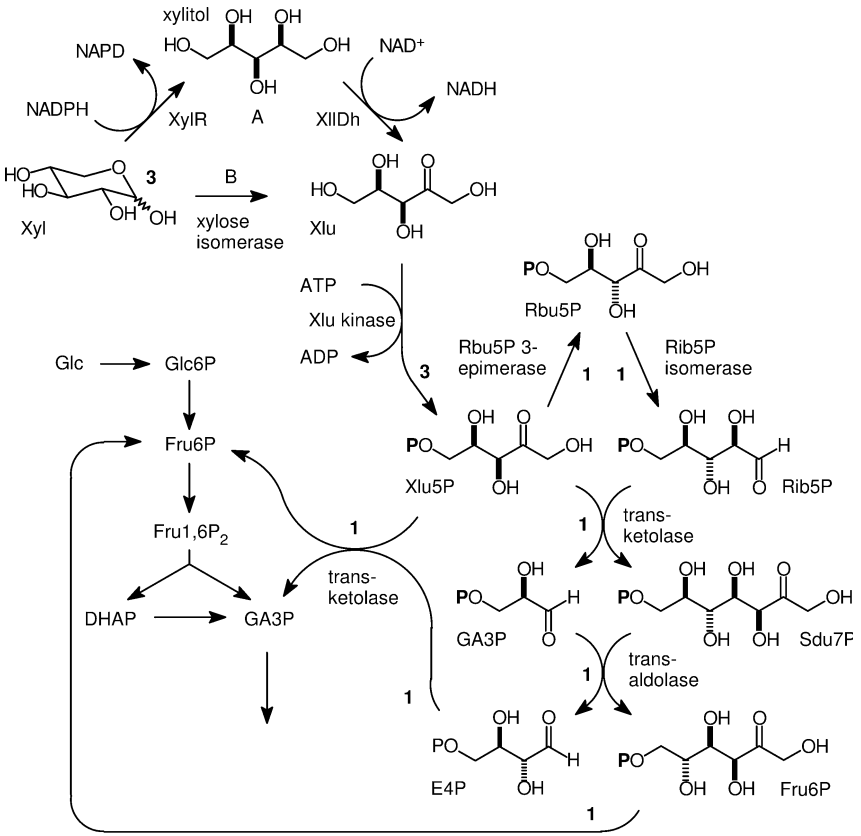


Fig. 8.5 Biochemical pathways for the conversion of xylose into xylulose (A: in yeasts; B: in bacteria) and the pentose phosphate pathway showing the idealized carbon flow in a xylose-fermenting organism. Compounds: E4P, D-erythrose-4-phosphate; Rib5P, D-ribose-5-phosphate; Rbu5P, D-ribulose-5-phosphate; Sdu7P, D-sedoheptulose-7-phosphate; Xlu, D-xylulose; Xlu5P: D-xylulose-5-phosphate; Xyl, D-xylose. Enzymatic activities: XylR, xylose reductase; XIIDH, xylitol dehydrogenase.

harbor a Xyl isomerase which converts Xyl into Xlu, but these organisms generally are inefficient ethanol producers.

Xlu is subsequently phosphorylated into xylulose-5-phosphate (Xlu5P), which is the entrance point into the pentose phosphate pathway. This latter metabolic pathway, which is interconnected with the upstream half of the glycolysis pathway (see Fig. 8.5), can metabolize Xlu5P into GA3P.

8.2.2.1 **Microbial Production of Ethanol**

Raw materials for the fermentation of ethanol are sugar molasses (Brazil), corn steep liquor and corn starch hydrolysate (USA). Industrial ethanol fermentation is highly developed and the stoichiometric yield can be as high as 1.9 mol mol^{-1} [25, 26]. The

STY is high for a fermentative procedure and ranges from 140 g L^{-1} d^{-1} for a continuous tank reactor to 1.2 kg L^{-1} d^{-1} in a continuous tower reactor with cell recycle. Depending on the ethanol tolerance of the production species, ethanol is produced to a concentration of 12–20%. The ethanol is traditionally recovered from the fermentation broth via an energy-intensive distillation step, but it is sought to replace the latter by pervaporation or reversed osmosis [25].

It has been predicted that lignocellulose conversion will, eventually, provide the cheapest route to ethanol [27], but its adoption has been delayed by the expensive pretreatment and the inefficient fermentation of the pentose-rich cellulose-derived sugars. Improvement has been sought via metabolic engineering in two ways. The first was to introduce the ethanol-forming enzymes PDC and ADH (see Fig. 8.4) in non-ethanologenic pentose-fermenting microbes (such as *Klebsiella oxytoca*). Alternatively, it has been attempted to engineer the capability to ferment pentoses in ethanol-producing yeasts, mainly *S. cerevisiae* and *Zymomonas mobilis* [24]. Both strategies were successful in principle, but the ethanol concentrations (<5%) remained undesirably low [24, 28].

This intractable problem may now be close to being solved. A *Saccharomyces* species that expressed the xylose isomerase gene from an anaerobic fungus was found to grow slowly on pentoses [29]. Improvement resulted from a combination of rational engineering – overexpression of the pentose phosphate-converting enzymes (see Fig. 8.5) – and classical strain improvement [30]. The authors conclude: "The kinetics of xylose fermentation are no longer a bottleneck in the industrial production of ethanol with yeast."

8.2.2.2 **Green Aspects**

A major part of the bioethanol is used as transport fuel, with the triple objectives to decrease the use of fossil fuels, reduce traffic-generated CO_2 emissions and improve the quality of the exhaust gas by adding an oxygenate to the fuel. The option to produce ethene from ethanol is still being considered. An energy analysis of the production of ethanol from corn [31] concluded that the energy gain (from sun to pump) ranges from 4% to 46% (taking the energy *input* as 100%). An energy gain of 75% would be possible by applying the best available technology in every step, which still means that 57% of the energy value of the output is required to drive the process.

In contrast, the energy gain of ethanol fermentation from a cellulose-based crop was estimated at only 10% [31]. A life cycle assessment of bioethanol from wood came to a similar conclusion [32]. This unsatisfactory outcome mainly results from the energy-intensive pretreatment with steam explosion, such as is used by Iogen [16]. The replacement of the latter by CO_2 explosion [33] may redress the energetic balance.

The unfavorable energy balance notwithstanding, the fermentation of ethanol, as well as other chemicals, from lignocellulose hydrolysate is considered highly desirable by some to avoid competition with food production. We note, however, that the large scale processing of waste lignocellulose runs counter to sensible

agricultural practice to return plant material to the soil for the preservation of long-term soil structure [16].

8.2.3 Lactic Acid

The industrial production of lactic acid [34, 35], which dates back to 1881, is undergoing a remarkable transition. Lactic acid used to be a fairly mature fine chemical that was produced, in the mid 1990s, at a volume of 50–70 kt a^{-1} worldwide. A major share (25 kt a^{-1}, including simple esters etc.) is used in the food industry.

The potential of lactic acid as a renewable, broadly applicable intermediate and a building block for green polymers, solvents and plasticizers was recognized over 10 years ago [36]. The excellent balance of properties of lactic acid polymers (polylactic acid, PLA) [37, 38] and copolymers would make these materials ideal renewable and biodegradable replacements for, e.g., polycarbonate and polystyrene. The production of PLA was too expensive for such large-scale applications, however, and its use remained limited to niche markets, such as surgical sutures. As lactic acid was generally regarded as a relatively mature fine chemical that lacked any incentive, until the mid-1980s, to obtain cost reductions through innovation, the price was kept high by a lack of high-volume applications and vice versa.

This is now changing, as a new and very big player has entered the field. Cargill (USA), which in the late 1990s started to push fermentatively produced lactic acid as an emerging commodity [39], has opened a production facility with a capacity of 140 kt a^{-1} of polylactic acid in 2002 [40]; a market potential of 500 kt a^{-1} of lactic acid products in 2010 is anticipated.

Lactic acid is produced chemically from acetaldehyde, by hydrocyanation, followed by acid hydrolysis of the cyanohydrin (Fig. 8.6 a). The crude lactic acid is purified via esterification with methanol, distillation of the ester and hydrolysis with recycling of the methanol [34]. Major drawbacks are the production of an equivalent of ammonium sulfate and the cumbersome purification procedure that is required to obtain food-grade lactic acid.

The fermentative production of lactic acid from carbohydrates has repeatedly been reviewed recently [36, 41, 42]. Two classes of lactic acid producers are discerned: the homofermentative lactic acid bacteria, which produce lactic acid as the sole product, and the heterofermentative ones, which also produce ethanol, acetic acid etc. [43]. Recently, the focus has been on (*S*)-L-lactic acid producing, homofermentative *Lactobacillus delbrueckii* subspecies [42].

The anaerobic fermentation of lactic acid is traditionally performed at up to 50 °C over 2–8 d at pH 5.5–6.5 (lactic acid bacteria are highly sensitive to acid). The pH is maintained by titration with a base, usually calcium carbonate. The product concentration is kept below approx. 100 g L^{-1} to prevent precipitation of calcium lactate, as the separation of a precipitate from the biomass would be too elaborate. The stoichiometric yields are high, of the order of 1.7–1.9 mol mol^{-1} (85–95% of the theoretical yield) but the space–time yield, which is ap-

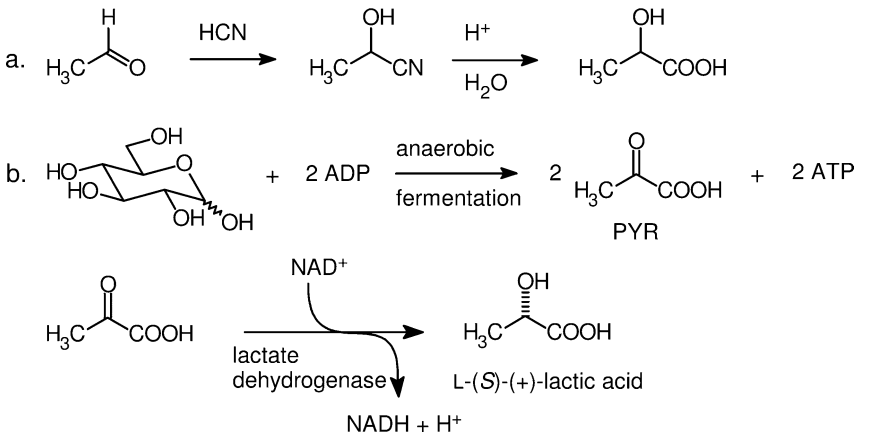


Fig. 8.6 Lactic acid and its production routes: (a) chemically, from acetaldehyde; (b) via fermentation.

prox. 100 g L^{-1} d^{-1}, is rather modest for a commodity and reflects the traditional nature of industrial lactic acid fermentation.

Acidification of the culture supernatant, filtration, treatment with active charcoal and concentration usually result in food-grade lactic acid, if the feedstock was pure glucose. Otherwise, more extensive purification may be required.

The fermentative process described above is not very atom-efficient – approx. 1 kg of calcium sulfate is formed per kg of lactic acid – and is expensive to operate due to the laborious DSP. It was good enough to compete, at times precariously, with the chemical manufacture of lactic acid [35] but extensive innovations were required to elevate lactic acid to commodity status.

The growing interest in renewable raw materials spurred, from the mid-1980s onwards, interest in the development of highly integrated, low-waste procedures for the fermentation, primary purification and processing of lactic acid. Continuous removal of lactic acid, via solvent extraction or otherwise, would obviate the need for adding base but is not feasible at pH 5.5–6.5, however, because only 2% of the lactic acid (pK_a 3.78) is uncharged at pH 5.5.

Acid-tolerant lactic acid bacteria would solve this latter problem and have indeed been obtained from screening [44] and via whole genome shuffling [45]. Alternatively, a lactate dehydrogenase gene could be inserted in a yeast, such as *S. cerevisiae*. These grow well at low pH and efficiently channel carbon into the glycolysis pathway under anaerobic conditions [46, 47]. Such constructs were indeed found to produce up to 55 g L^{-1} of lactic acid at pH 3.6 [46].

When the initial shortcomings of these engineered yeasts, such as low stoichiometric yield and productivity, have been ironed out, it may be expected that lactic acid fermentation in an acidic medium, combined with solvent extraction of the product, will evolve into a procedure of unprecedented efficiency.

Alternatively, the fermentation can be performed at pH 5.5–6.5 while employing a salt-free procedure to convert lactate into lactic acid [48]. Cargill has pat-

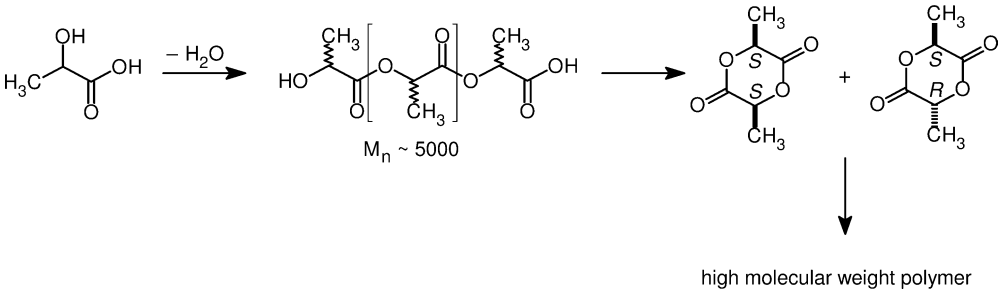


Fig. 8.7 Schematic representation of the solventless conversion of lactic acid into PLA.

ented a procedure, comprising acidification of the culture supernatant with CO_2 and extraction of the lactic acid into an organic phase [49], that may have been adopted.

Highly integrated procedures have been developed to convert lactic acid into dilactides and subsequently into PLA [38b, 38c, 50]. In the Cargill process, the aqueous lactic acid, which contains a preponderance of the (*S*)-enantiomer, is condensed into a mixture of oligomers, which is subsequently converted, in the presence of a tin catalyst [50], into the (*S,S*)- and (*R,S*)-dilactides (see Fig. 8.7) [51]. These are separated by vacuum distillation to tune (*S,S*)/(*R,S*) to the desired properties of the polymer [38c]. Subsequently, the lactide is subjected to ring-opening polymerization in the presence of a tin catalyst, such as tin octanoate, in a solventless procedure [38b, 50]. Major strengths of the procedure are the removal of water early in the procedure, the efficient separation of the dilactide diastereoisomers and the absence of solvents. The PLA is to be marketed under the trade names of Nature Works™ PLA by Cargill Dow Polymers and LACEA by Mitsui (Japan) [38c]. If the current price of lactic acid ($2 kg^{-1}) is translated into $3 kg^{-1} for PLA, the latter product should be able to compete with polycarbonate at $4.4 kg^{-1} [52]. If plans to produce lactic acid from cellulose hydrolysate [16] come to fruition, PLA could even become competitive with polystyrene ($2 kg^{-1}).

8.2.4
1,3-Propanediol

1,3-Propanediol (1,3PD) is also undergoing a transition from a small-volume specialty chemical into a commodity. The driving force is its application in poly(trimethylene terephthalate) (PTT), which is expected to partially replace poly(ethylene terephthalate) and polyamide because of its better performance, such as stretch recovery. The projected market volume of PTT under the tradenames CORTERRA (Shell) and Sorona™ 3GT (Dupont) is 1 Mt a^{-1} within a few years. In consequence, the production volume of 1,3PD is expected to expand from 55 kt a^{-1} in 1999 to 360 kt a^{-1} in the near future. 1,3PD used to be synthesized from acrolein by Degussa and from ethylene oxide by Shell (see Fig. 8.8) but a fermentative process is now joining the competition.

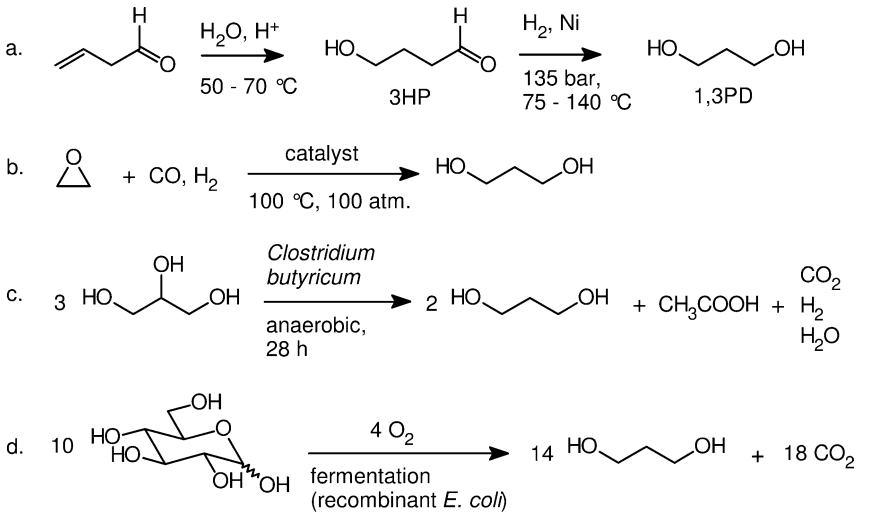


Fig. 8.8 Processes for 1,3PD: (a) from acrolein (Degussa); (b) from ethylene oxide (Shell); (c) from glycerol, via anaerobic fermentation (Henkel); (d) from glucose, using an *E. coli* cell factory (Dupont-Genencor). Compounds: 3HP, 3-hydroxypropanal; 1,3PD, 1,3-dihydroxypropane.

The Degussa process (now owned by Dupont) starts from acrolein, which is hydrated in the presence of an acidic ion exchanger into 3-hydroxypropanal (3HP, Fig. 8.8a). The latter is subsequently extracted into isobutyl alcohol and hydrogenated over a Ni catalyst [53]. The overall yield does not exceed 85%, due to competing water addition at the 2-position and ether formation in the initial step. It has been announced that Degussa will supply up 10 kt a^{-1} to Dupont until the fermentative process of the latter company (see below) comes on stream [54].

Shell produces 1,3PD from ethylene oxide via hydroformylation with synthesis gas (Fig. 8.8b). The transformation required two separate steps in the past [55], but has been improved [56], which made the large-volume use of 1,3PD in poly(trimethylene terephthalate) economically viable, and the two steps have been telescoped into one [57, 58]. Shell has a capacity to 70 kt a^{-1} [59].

The fermentation of 1,3PD from glycerol (see Fig. 8.8c) was discovered in the late 19th century [60]. It has since been found that a considerable number of bacteria can use glycerol as a source of carbon and energy under anaerobic conditions and the reaction pathways have been elucidated [61]. Out of every three molecules of glycerol, one is oxidized, phosphorylated into DHAP and subsequently metabolized via the glycolysis pathway and the TCA cycle; the other two are converted into 1,3PD, to maintain redox balance (see Fig. 8.9), via dehydration and NADH-driven reduction.

There has been a long-standing interest in the possible microbial production of 1,3PD from glycerol in *Citrobacter, Klebsiella* and *Clostridia* species [61–63], to

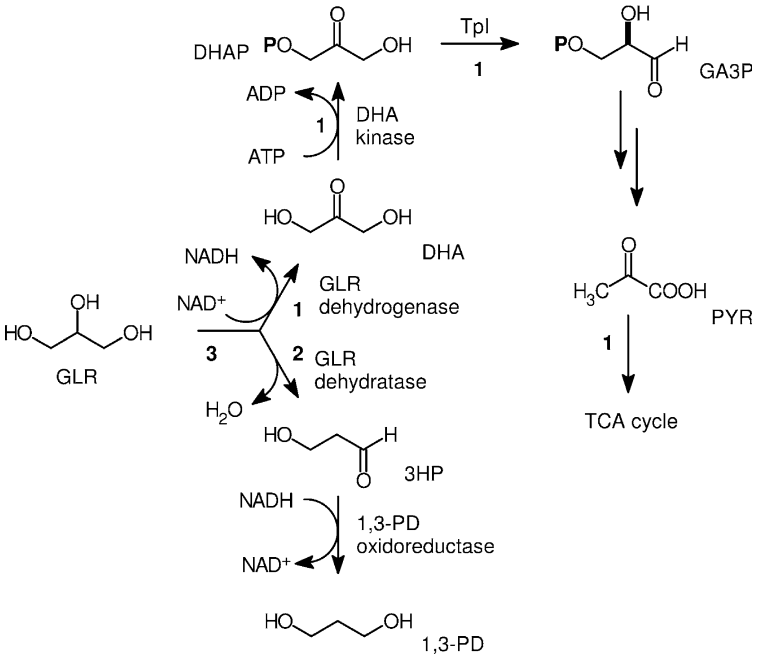


Fig. 8.9 Anaerobic fermentation of glycerol [61].
Compounds: GLR, glycerol; DHA, dihydroxyacetone.

relieve a projected oversupply of glycerol. The stoichiometric yields are close to the theoretical maximum of 0.67 mol/mol but the product titer has remained limited to 70–78 g L^{-1} (STY 75 g L^{-1} d^{-1}), in spite of metabolic pathway engineering studies [61].

Genencor has taken a radically different approach [22] by engineering the central metabolism of *E. coli* for the production of 1,3PD from glucose [64]. To this end, the enzymes from *S. cerevisiae* that convert DHAP into glycerol (see Fig. 8.4), as well as the glycerol dehydratase complex from *K. pneumonia*, have been cloned into the production organism. Hence, the latter combines functionalities that in Nature require two very different organisms. The final reduction step, of 3HP into 1,3PD, is taken care of by a non-specific NADPH-dependent alcohol dehydrogenase that is native to *E. coli*.

Efficiency required that all glycerol should flow into the 1,3PD pathway. Hence, glycerol was prevented from re-entering the central metabolism [64]. A second, more radical modification was made to the glucose uptake and phosphorylation mechanism, which in *E. coli* natively occurs via the PEP-consuming phosphate transfer system (PTS) with formation of PYR, which enters the oxidative branch. The consumption of 1 mol of PEP per mol of glucose puts an artificial ceiling on the stoichiometric yield of 1.0 mol mol^{-1}. Replacing the PTS by an ATP-dependent system [64] effectively removed the restriction of the product yield.

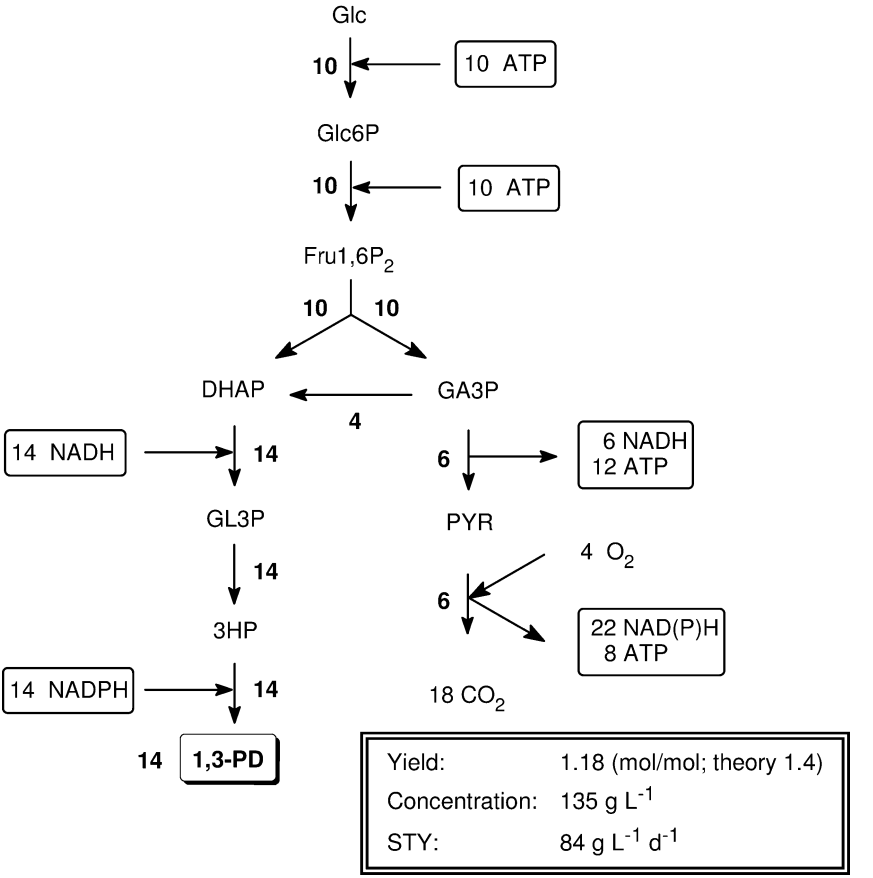


Fig. 8.10 Production of 1,3PD in an *E. coli* cell factory [64].

As shown in Fig. 8.10, energy and redox equivalents are required to drive the transformation of glucose into 1,3PD. These are provided by converting some of the glucose all the way into CO_2, which restricts the stoichiometric yield on glucose to 1.4 mol mol^{-1} maximum. A stoichiometric yield of 1.18 mol mol^{-1} of 1,3PD (50% by weight) has been obtained in practice [64], which corresponds with 85% of the theoretical yield, at a product concentration of 135 g L^{-1}.

It should be noted that 1,3PD represents only 36% of the molecular mass of the polymer; hence, if the fermentative route to 1,3PD is the winner its contribution to sustainability still remains limited. Unless, of course, the terephthalic acid building block also is replaced by a renewable one, such as furan-1,5-dicarboxylic acid (see Section 8.3.5).

8.2.5
3-Hydroxypropanoic Acid

3-Hydroxypropanoic acid (3HPA) is under development as a future platform chemical and monomer derived from biomass. It is, at the present time, not produced on an industrial scale, either chemically or biotechnologically. 3HPA could be a key compound for the production of biomass-derived C_3 intermediates, such as acrylic acid, acrylic amide and malonic acid (see Fig. 8.11). Hydrogenation of 3HPA would provide a competing procedure for the production of 1,3PD (see Section 8.2.4) that could be more economical than the DuPont and Shell processes [65].

There is no microbe known to produce 3HPA. Various metabolic routes from glucose to 3HPA can be designed [66] but only two of these, via lactic acid and 3-aminopropionic acid, respectively (Fig. 8.12), have been developed in any depth, mainly by Cargill. The lactic acid route has two major disadvantages: it passes through a local energy maximum (acrylCoA) and there is hardly any driving force because the heats of formation and ionization constants of lactic acid and 3HP are very close [67].

The alternate route, via pyruvic acid and 3-aminopropanoate, is exothermic for nearly the whole way and is anaerobic with production of ATP. The transformation of L-alanine into 3-aminopropanoate is a major hurdle, as this activity is not known in Nature and was not found upon screening. A corresponding lysine mutase is known, however, and was engineered into an alanine mutase, in collaboration with Codexis [67, 68]. The selection method was based on the disruption of the *panD* gene (see Section 8.2.9), which puts the organism under selective pressure to evolve an alternative route to 3-aminopropionate to satisfy its requirement for (*R*)-pantoic acid. Furthermore, all competing NAD^+-forming

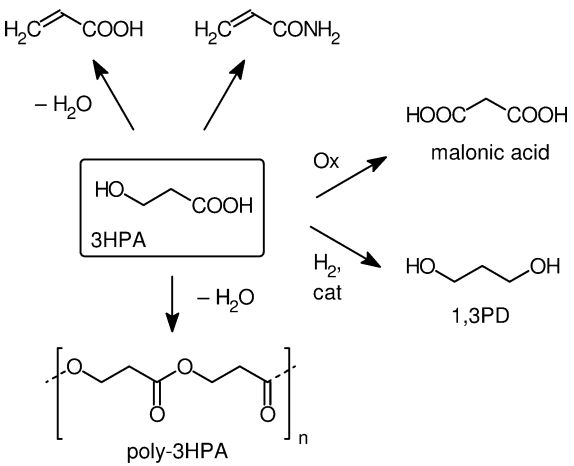


Fig. 8.11 3-Hydroxypropanoic acid as a potential platform chemical. Compound: 3HPA, 3-hydroxypropanoic acid.

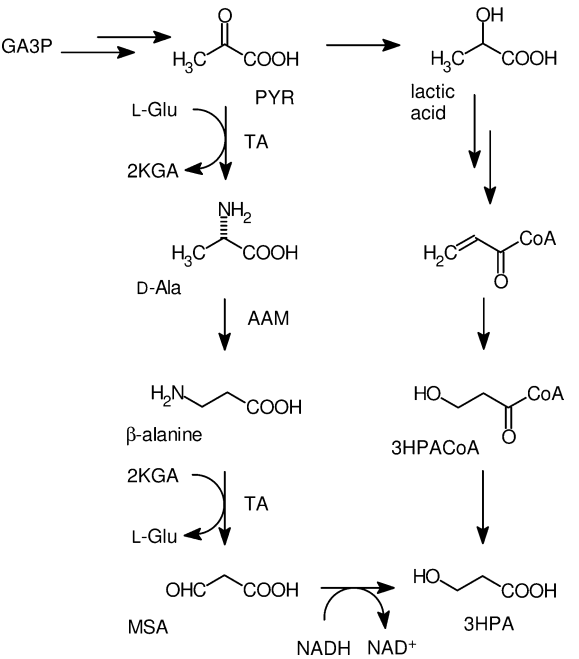


Fig. 8.12 Microbial production routes to 3-hydroxypropanoic acid. Compounds: 3HPCoA, 3-hydroxypropanoyl coenzyme A ester; 2KGA, 2-ketogluconic acid; MSA, malonate semi-aldehyde. Enzymatic activities: AAM, alanine-2,3-aminomutase; TA, transaminase.

pathways, such as production of ethanol, acetate, lactate etc., were switched off to force the organism into producing 3HPA to maintain redox balance [67]. Cargill has indicated that commercialization of the microbial production of 3HPA is still some years away.

8.2.6
Synthesizing Aromatics in Nature's Way

Microbes and plants synthesize aromatic compounds to meet their needs of aromatic amino acids (L-Phe, L-Tyr and L-Trp) and vitamins. The biosynthesis of these aromatics [69] starts with the aldol reaction of D-erythrose-4-phosphate (E4P) and phosphoenolpyruvate (PEP), which are both derived from glucose via the central metabolism, into DAHP (see Fig. 8.13). DAHP is subsequently converted, via a number of enzymatic steps, into shikimate (SA) and eventually into chorismate (CHA, see later), which is the common intermediate in the biosynthesis of the aromatic amino acids [70] and vitamins.

DAHP synthase combines E4P and PEP, which are both derived from the central metabolism (see Figs. 8.4 and 8.5 for more details). The biosynthesis of

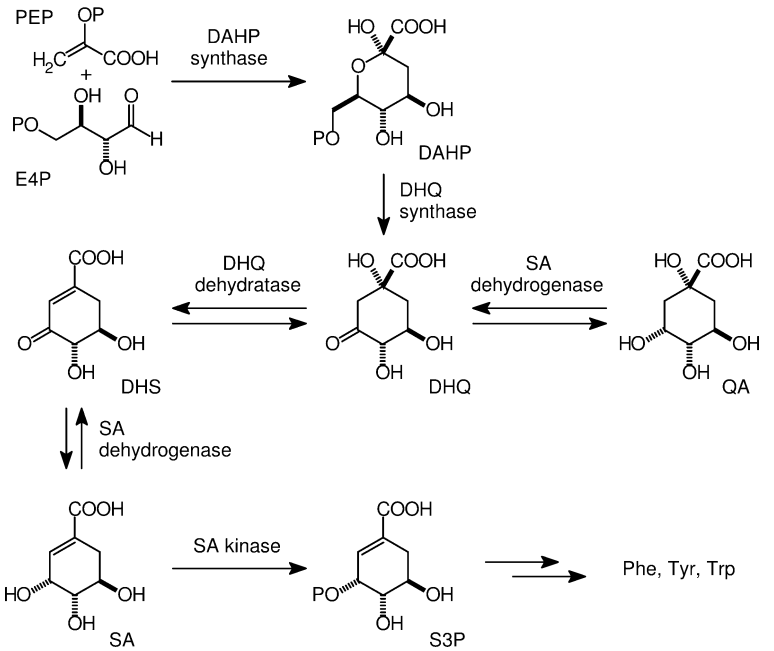


Fig. 8.13 Biosynthesis of aromatic compounds in *E. coli* [71]. Compounds: E4P, D-erythrose-4-phosphate; PEP, phosphoenolpyruvate; DAHP, 3-deoxy-D-*arabino*-heptulosonate-7-phosphate; DHQ, 3-dehydroquinate; QA, quinate; DHS, 3-dehydroshikimate; SA, shikimate; S3P, shikimate-3-phosphate.

E4P and PEP has been studied in much detail; the extensive mutagenesis that has been applied to increase the bioavailability of these latter compounds has been reviewed [70–72]. It should be noted that the PTS, which mediates the uptake and phosphorylation of glucose, limits the yield of DAHP from glucose at 0.43 mol mol^{-1} in a situation that is similar to that described above in Section 8.2.4. If all PEP could be channeled into the aromatic pathway the stoichiometric yield of DAHP could be as high as 0.86 mol mol^{-1}, at least in theory [73]. Various approaches to PEP conservation have been demonstrated [72, 74, 75].

The common pathway is regulated by several mechanisms, depending on the organism. In *E. coli*, which is the best investigated organism in this respect, metabolic engineering usually starts with the introduction of a feedback resistant DAHP synthase gene [70]. Overexpression of the subsequent enzymes in the common route, DHQ synthase and SA dehydrogenase, alleviates the kinetic bottlenecks caused by product inhibition.

SA has recently emerged as the preferred intermediate in an industrial process for the antiviral medicine Tamiflu® [76]. Because SA is only (sparingly) available from the fruit of the *Illicium* plants, there is much interest in its microbial production, mainly in rationally designed *E. coli* mutants [77].

An *E. coli* in which further conversion of SA was blocked by disruption of SA kinase, combined with mutagenesis for increased SA production, excreted SA into the culture medium [78]. 27 g L^{-1} of SA was produced, accompanied by DHS (4.4 g L^{-1}) and QA (12.6 g L^{-1}) [78]. This latter compound, which is particularly troublesome in the subsequent purification of SA, arises from the conversion of DHQ into QA, due to incomplete selectivity of SA dehydrogenase. The formation of QA could eventually be suppressed by performing the culture under glucose-rich conditions [79]. When combined with PEP conservation, SA was produced at a level of 84 g L^{-1} in a fermentation on the 10 L scale, which corresponds with a yield of 0.33 mol mol^{-1} glucose [75], along with DHS (10 g L^{-1}) and QA (2 g L^{-1}). Hence, the total yield on hydroaromatic products was 0.38 mol mol^{-1}.

There also is some interest in QA as a chiral building block and a food acidulant. The compound is not naturally produced by *E. coli* but, as noted above, an *E. coli* engineered for the production of SA fortuitously produced modest amounts of QA [78, 79]. A very similar mutant, which lacked DHQ dehydratase (see Fig. 8.13), produced QA in copious amounts (49 g L^{-1}), along with a small amount (3.3 g L^{-1}) of DHQ [80].

The Frost group [80, 81] has been particularly active in devising microbial and chemical-microbial procedures for the benzene-free synthesis of phenolic compounds from glucose via the hydroaromatic compounds DHS, QA and SA. As has been noted previously, such products can be synthesized more cheaply from coal via the methanol route, if oil should become too expensive [82].

The same group has demonstrated the microbial synthesis of vanillin in an *E. coli* mutant [83] but the yield and titer (6.2 g L^{-1}) were too low to be of immediate practical value. A completely microbial route to gallic acid [84], which is currently isolated from natural sources [85], produced 20 g L^{-1} of the desired product in the presence of considerable amounts of side-products [84].

8.2.7
Aromatic α-Amino Acids

The market volume of *L-phenylalanine* (L-Phe) has rapidly increased in recent years to 14 kt in 2002, according to an authorative estimate [86], which is mainly due to the commercial success of the artificial sweetener aspartame. L-Phe has been produced by a number of chemoenzymatic routes in the past [87] (see Fig. 8.14) and many more have been developed [88] but never commercialized. Fermentation of L-Phe is now so efficient that it has rendered all of the chemoenzymatic procedures obsolete.

L-Phe can be prepared via the enantioselective hydrolysis of *N*-acetyl-D,L-Phe and microbial reductive amination of phenylpyruvate (see Fig. 8.14) [87]. The stoichiometric yields of these processes were high but the precursors required 3–4 synthetic steps from the basic starting materials in most cases. The phenylammonia lyase route, in contrast [89], provided L-Phe in only two steps from the basic chemicals benzaldehyde and acetic anhydride [90]. The enzymatic step

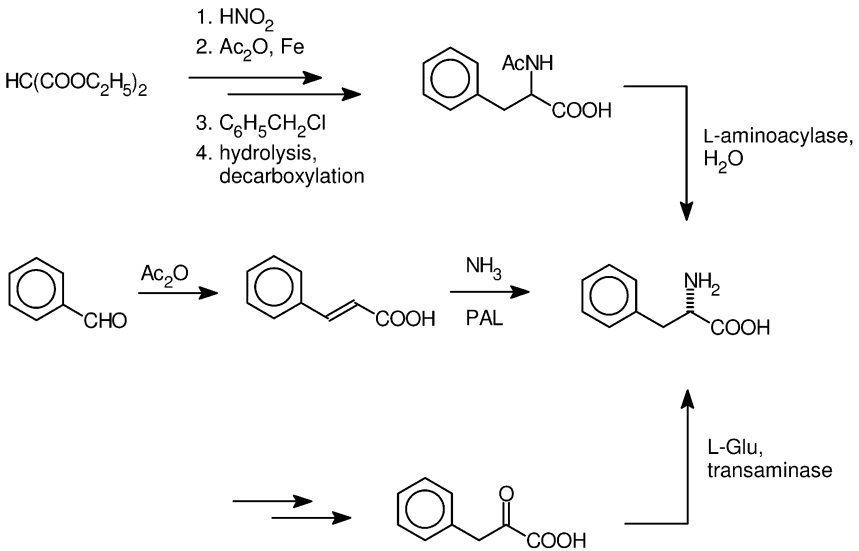


Fig. 8.14 Chemoenzymatic processes for L-phenylalanine.

afforded up to 50 g L^{-1} of L-Phe in 83% yield [89c]; an STY of 34 g L^{-1} d^{-1} has been claimed [89b]. The procedure was used to provide the L-Phe for the first production campaign of aspartame by Searle [87].

The biosynthetic pathway from SA into L-Phe [69, 70] is shown in Fig. 8.15. The synthesis of chorismate (CHA), the common intermediate in the biosynthesis of the aromatic amino acids, requires an extra equivalent of PEP, which limits the yield of L-Phe from glucose to 0.30 mol mol^{-1} if PEP is not conserved [91]. The further transformation of CHA into phenylpyruvic acid (PPY) suffers from inhibition by L-Phe and is also subject to transcriptional control [69, 92]. The final step is a reductive amination of PPY into L-Phe with consumption of L-Glu.

In the early years, L-Phe was microbially produced with *Corynebacterium glutamicum* and *E. coli* strains which had been deregulated with respect to the end product via classical strain improvement. More recently, metabolic engineering has been employed to address nearly all aspects of the biosynthesis of L-Phe; the work has been reviewed [70, 93].

Little is known about the current production levels of L-Phe, but in the early days of metabolic engineering 50 g L^{-1} of L-Phe, with a yield on glucose of 0.27 mol mol^{-1}, was produced by an engineered *E. coli* [92]. This is near the theoretical limit [91] and one would surmise that in the starting species used by these authors some PEP-conserving mutations had been introduced via classical strain improvement.

Few details have been disclosed on the DSP of L-Phe fermentation, which may indicate that it is fairly traditional. A procedure for integrated product removal, which also resulted in increased productivity and yield on glucose, has recently been published [94].

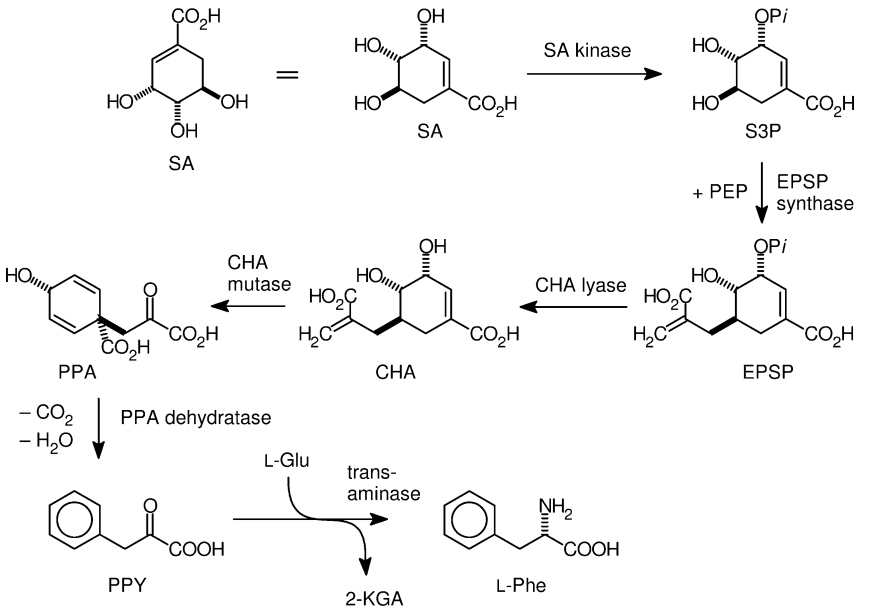


Fig. 8.15 Biosynthesis of L-Phe. Compounds: EPSP, 5-*enol*-pyruvoylshikimic acid-3-phosphate; CHA, chorismic acid; PPA, prephenic acid; PPY, phenylpyruvic acid; S3P, shikimic acid 3-phosphate.

The fermentation of L-Phe has obviously emerged victorious, on purely economic grounds and is now so efficient that even D,L-Phe, which is used by the Holland Sweetener Company in the enzymatic production of aspartame, is obtained by racemization of L-Phe rather than via a chemical procedure.

L-Tryptophan (L-Trp) was produced, mainly by Japanese companies, on a scale of 500–600 t a^{-1} in 1997 [70]. It is an essential amino acid that is used as a food and feed additive and in medical applications. L-Trp is, at US$ 50 kg^{-1} (feed quality), the most expensive aromatic amino acid and it is thought that the market for L-Trp could expand drastically if the production costs could be brought down. There is no chemical process for L-TrP and enzymatic procedures starting from indole, which were very efficient, could not compete with fermentation [95]. L-Trp has been produced by precursor fermentation of anthranilic acid (ANT, see Fig. 8.16), but the serious effects of minor by-products caused the process to be closed down. Since the mid-1990s all L-Trp is produced by *de novo* fermentation.

The biosynthesis of L-Trp from CHA is outlined in Fig. 8.16. The complex transformation of CHA into phosphoribosyl anthranilate (PRAA) is, in *E. coli* and *C. glutamicum*, catalyzed by a protein aggregate that is inhibited by the L-Trp end product. The final two steps are performed by a single protein; indole, which is toxic to the cell, is channeled directly into the active site where the fi-

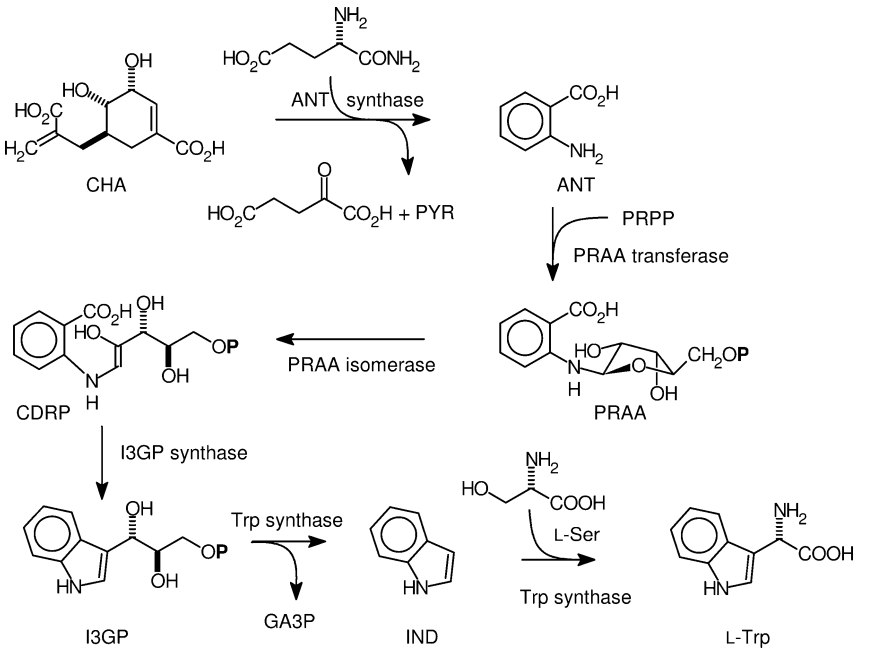


Fig. 8.16 Biosynthesis of L-Trp. Compounds: ANT, anthranilate; CDRP, 1-(*o*-carboxyphenylamino)-1-deoxyribulose-5-phosphate; I3GP, indole-3-glycerolphosphate; IND, indole; PRAA, phosphoribosyl anthranilate; PRPP, 5-phosphoribosyl-*α*-pyrophosphate.

nal conversion takes place [69]. The genes that code for the Trp pathway are tightly clustered on the *trp* operon, which is subject to transcriptional control by a single repressor protein.

In comparison with L-Phe, extra glucose is required to provide the ribose phosphate derivative and the maximum yield of L-Trp from glucose, without PEP-conserving modifications, has been estimated at 0.20 mol mol^{-1} [96].

C. glutamicum and *E. coli*, which share very similar biosynthetic pathways and control architectures, have been subjected to pathway engineering for the production of L-Trp. Modifications that have been reported include the, now familiar, feedback-resistant DAHP synthase and the enzymes in the Trp pathway were freed from regulation [72, 97] and overexpressed.

A shortage of L-Ser, which caused accumulation of indole and cell death, was remedied by overexpression of the first enzyme in the Ser pathway [98]. Additionally, engineering of the central metabolism to increase the availability of E4P (see Fig. 8.5) increased the production of L-Trp in *C. glutamicum* [99].

The best published L-Trp production levels with engineered *C. glutamicum* and *E. coli* species range from 45–58 g L^{-1} (STY 17–20 g L^{-1} d^{-1}) [72, 99]; a 0.2 mol mol^{-1} yield on Glc, which is the theoretical maximum, has been reported [100].

8.2.7
Indigo: the Natural Color

The blue indigo dye has been used from prehistoric times onwards [101]. Traditionally, it was prepared from plants, such as *Indigofera tinctoria* (in the East) or *Isatis tinctoria* (woad, in Europe). These plants contain derivatives of indoxyl (see Fig. 8.17) from which the latter was liberated by laborious fermentation. Spontaneous aerobic oxidation of indoxyl yields indigo; the reaction seems to proceed via leucoindigo and generates 1 mol of H_2O_2 per mol of indoxyl oxidized [102]. Reduction of the insoluble indigo, by various methods, yields the soluble and colorless leucobase, which is applied to the textile and is oxidized back to indigo upon exposure to air.

A chemical process for indigo, developed by BASF in the late 19th century, rapidly pushed the natural material from the market [103]. Nowadays, 17 kt a^{-1} of indigo is produced chemically; most of this output is used to dye the 10^9 blue jeans that are produced annually. Amgen and later Genencor have been developing a fermentative process for indigo since the early 1980s. Remarkably, the EC now supports a research project that aims at a revival of the agricultural production of indigo [104].

Nearly all indigo is produced from *N*-phenylglycine (see Fig. 8.18) via fusion with potassium and sodium hydroxide, followed by treatment with sodamide [103]. The melt containing the dialkalimetal salt of indoxyl is subsequently dissolved in water, and indigo is formed by aerobic oxidation. Filtration and wash-

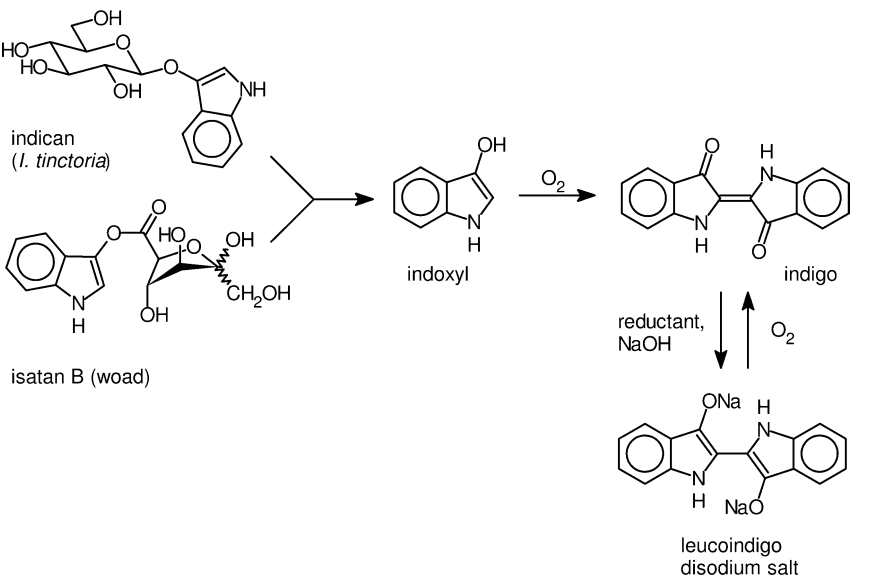


Fig. 8.17 Indigo, its basic chemistry and its natural sources.

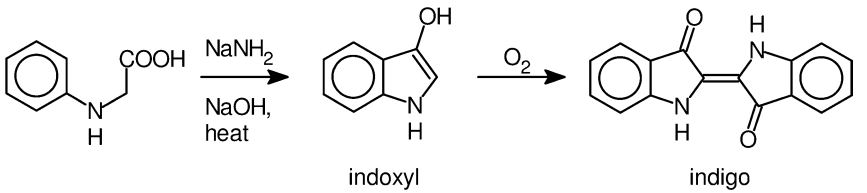


Fig. 8.18 Indigo production via the Heumann-Pfleger process (BASF).

ing affords nearly pure indigo; the alkaline filtrate is concentrated, filtered to remove side-products and recycled [103].

The key to an industrially viable microbial synthesis of indigo, which already had been demonstrated in principle in 1927 [105], was the discovery that naphthalene dioxygenase (NDO) from *P. putida* was able to oxidize indole into the indole precursor *cis*-2,3-hydroxy-2,3-dihydroindole (see Fig. 8.19) [106]. Genencor has disclosed details of a prototype indigo-producing *E. coli* that is based on its L-Trp producing strain [107]. This latter species carried the genes for a feedback-resistant DAHP synthase (see Fig. 8.11) and ANT synthase to maximize the carbon flow into the Trp pathway. The Trp synthase was engineered to allow indole to escape from the enzyme complex, which does not occur normally [69]; the insertion of a *P. putida* gene for naphthalene dioxygenase resulted in the production of *cis*-2,3-dihydroxyindole (DHI), which reacts extracellularly into indigo (Fig. 8.19).

The indigo production was 50% lower than expected on the basis of the Trp productivity of the parent strain [107]. This latter problem was traced to inactivation of DAHP synthase by exposure to indoxyl or its oxidation products; it was also found that PEP exerted a protecting effect on DAHP synthase. The production of indigo was improved to approx. 18 g L^{-1} (6.2 g L^{-1} d^{-1}) by increasing the gene dosage for DAHP synthase and mutations that increased the bioavailability of PEP [107].

A problem connected with the microbial production of indigo is the formation of a small amount of isatin, which could be partly suppressed by adjusting the O_2 concentration [108]. Isatin reacts with indoxyl to give indirubin, which gives the finished denim an undesirable red cast. The introduction of an isatin-degrading enzyme (isatin hydrolase from *P. putida*) satisfactorily reduced the level of indirubin [107].

Subsequently, indigo was successfully produced via fermentation on the 300 000 L scale at a cost that was comparable with the price of chemical indigo [109]. Commercialization proved elusive, however, presumably because chemical indigo is marketed with a substantial profit margin. We note that it is common experience that the *total* costs of a new process, to compete, must be equal to (or lower than) the *production* cost of the existing process. In this particular case, it would seem that the STY of the fermentative process is too low to compete with a chemical procedure that encompasses only three steps from the basic chemicals aniline and acetic acid.

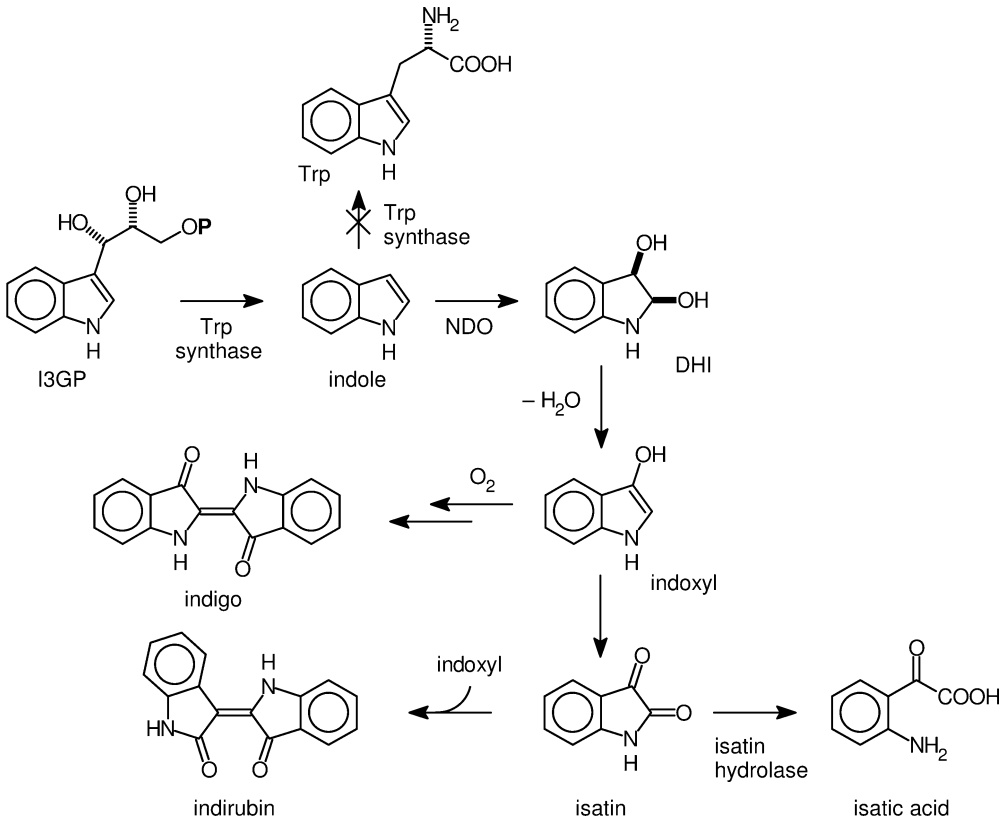


Fig. 8.19 Microbial production of indigo by an *E. coli* cell factory. Abbreviations: DHI, *cis*-2,3-dihydroxy-2,3-dihydroindole; NDO, naphthalene dioxygenase.

8.2.8
Pantothenic Acid

(*R*)-Pantothenic acid (vitamin B_5) is synthesized by microbes and plants, but not by mammals, who require it as a nutritional factor. Only the (*R*)-enantiomer is physiologically active. (*R*)-Pantothenic acid is produced as its calcium salt on a 6 kt a^{-1} scale, 80% of which is applied as an animal feed additive; major suppliers are Roche, Fuji and BASF. Pantothenic acid is produced via chemical methods [110] but a fermentative procedure has recently been commercialized.

The major industrial route to calcium pantothenate starts from isobutyraldehyde, which is condensed with formaldehyde. Hydrocyanation and hydrolysis affords the racemic pantolactone (Fig. 8.20). The resolution of pantolactone is carried out by diastereomeric crystallization with a chiral amine, such as (+)-2-aminopinane (BASF), 2-benzylamino-1-phenylethanol (Fuji) or (1*R*)-3-*endo*-aminonorborneol (Roche). The undesired enantiomer is racemized and recycled.

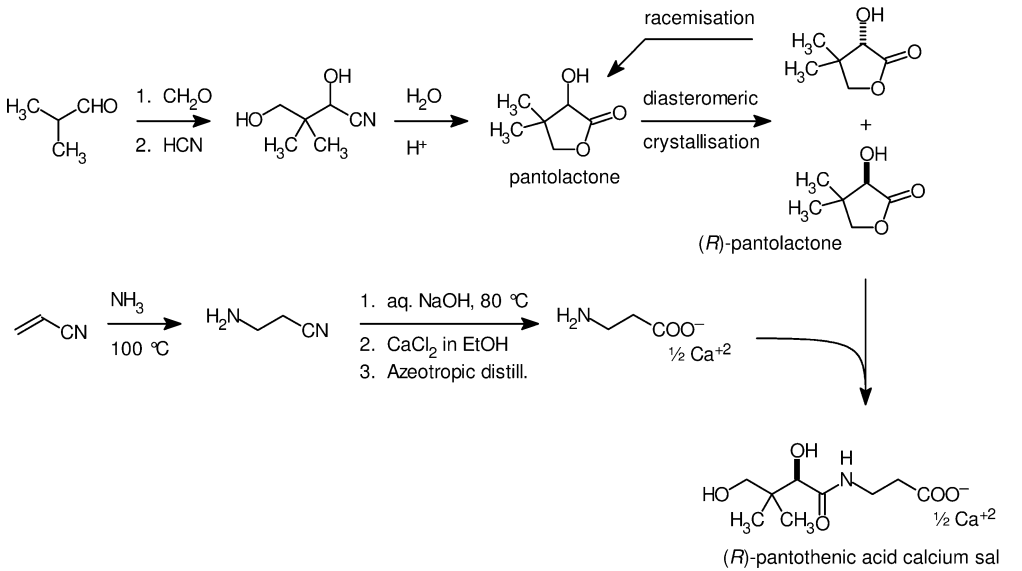


Fig. 8.20 Chemical production of (*R*)-pantothenic acid.

The reaction of (*R*)-pantolactone with calcium 3-aminopropionate (synthesized from acrylonitrile, see Fig. 8.20) affords calcium pantothenate [111].

The diastereomeric crystallization of pantolactone is laborious due to the need to recycle the resolving agent. Various schemes to replace this latter step with the chemical or microbial oxidation of pantolactone, followed by microbial reduction to the (*R*)-enantiomer, were unsuccessful because the productivity of the microbial step remained too low [110a].

The enantioselective hydrolysis of pantolactone into (*R*)-pantoic acid and (*S*)-pantolactone (Fig. 8.21), in the presence of (*R*)-pantolactone hydrolase from *Fusarium oxysporum* [110b], offers a better alternative. An alginate-entrapped

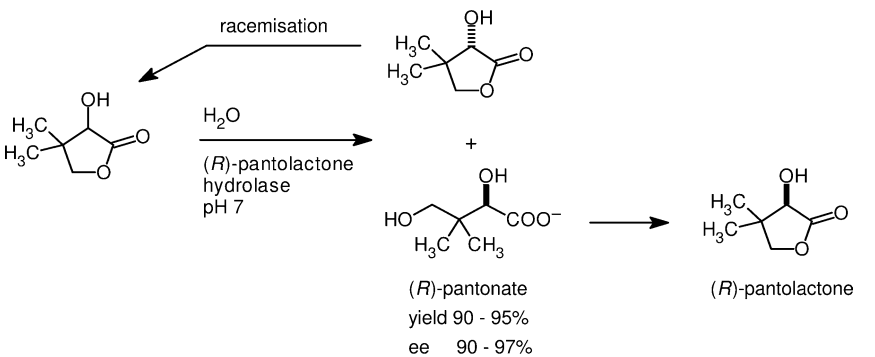


Fig. 8.21 Enzymatic resolution of pantolactone.

whole-cell preparation maintained its activity for 180 hydrolysis cycles at 350 g L^{-1} pantolactone. The enzymatic resolution of pantolactone does not, however, remedy the major drawback of the traditional production of pantothenic acid, which is the laborious purification of (*R*)-pantolactone.

The biosynthesis of (*R*)-pantothenate in *E. coli* [112] (see Fig. 8.22) and *Corynebacterium glutamicum* [113] has been elucidated. 3-Methyl-2-oxobutyrate, an intermediate in the L-Val biosynthesis pathway, is successively hydroxymethylated and reduced to (*R*)-pantoate. The latter intermediate is coupled, in an ATP-requiring reaction, with 3-aminopropionate that is derived from L-aspartate via decarboxylation. The corresponding genes have been identified [114].

(*R*)-pantothenic acid is an obvious candidate to be produced via fermentation, because all microorganisms synthesize the vitamin to meet their own requirements. Takeda Chemical Industries has developed a microbial partial synthesis of (*R*)-pantothenate in an *E. coli* mutant with enhanced expression of the *panB*, *panC* and *panD* genes [115]. High levels of (*R*)-pantothenate, 60 g L^{-1} [116], which corresponds with 30 g L^{-1} d^{-1}, were obtained when 3-aminopropionate was fed to the culture. Presumably, fermentation of (*R*)-pantothenate with suppletion of 3-aminopropionate is used by Degussa in the production of Biopan®.

Attempts at *de novo* fermentation of (*R*)-pantothenate in organisms such as *E. coli* [117], *Bacillus* species [118] and *C. glutamicum* [113, 119] met with low production levels of 1–2 g L^{-1} [113], which is too low to be of immediate practical value.

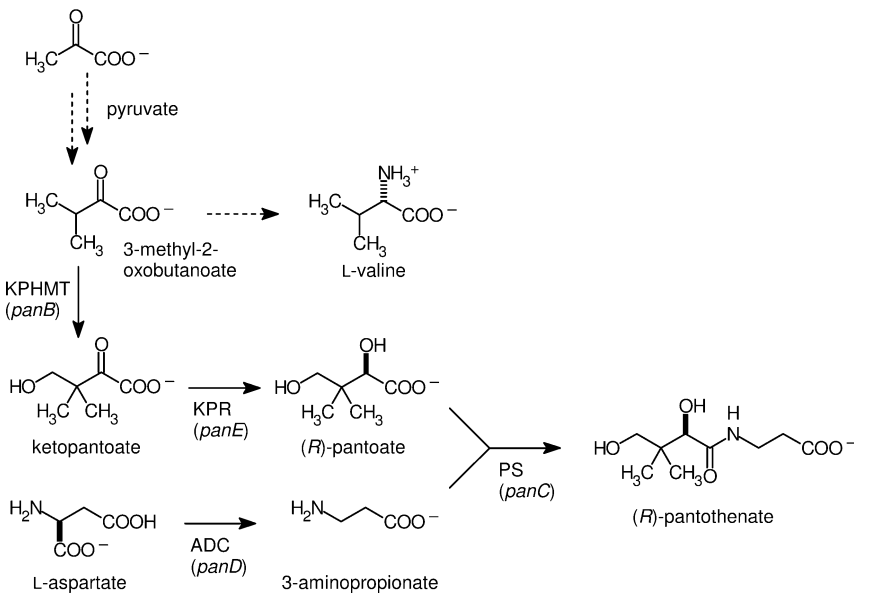


Fig. 8.22 The biosynthesis of (*R*)-pantothenate in *E. coli* [112]. Enzymatic activities: ADC, L-aspartate-1-decarboxylase; KPHM, α-ketopantoate hydroxymethyltransferase; KPR, α-ketopantoate reductase; PS, pantothenate synthase.

In conclusion, at least one producer of (*R*)-pantothenate is operating a fermentative process, with suppletion of 3-aminopropionate. Total microbial synthesis is much more challenging but, considering the pace of metabolic engineering, there is little doubt that one will be commercialized in the near future. Alternatively, the 3-aminopropionate could be derived from L-Ala, using the cellular machinery developed for the production of 3HPA (see Section 8.2.5) [67, 68].

8.2.9
The β-Lactam Building Block 7-Aminodesacetoxycephalosporanic Acid

Penicillin G is produced on a scale of >20 kt a^{-1} via fermentation in *Penicillium chrysogenum*; there is no competition from chemistry as production by chemical means would be far too elaborate. A relentless competition has driven the continuous improvement of penicillin G production [120]. Performance figures of the penicillin G culture are well-kept secrets but can be estimated at 15–20 g L^{-1} d^{-1}.

Penicillin G as well as approx. 4 kt a^{-1} of penicillin V are enzymatically hydrolyzed into the β-lactam nucleus 6-aminopenicillanic acid (6-APA), which is the building block for the semisynthetic penicillin antibiotics ampicillin and amoxycillin [121] (see Fig. 8.23). Part of the penicillin G is converted into 7-aminodesacetoxycephalosporanic acid (7-ADCA, 3 kt a^{-1}), which is the intermediate for the semisynthetic cephalosporins cephalexin and cefadroxil [122]. 7-ADCA has traditionally been produced from penicillin G via a chemical ring expansion but an all-bio process has recently been started up by DSM.

The chemical process for 7-ADCA dates back to the early days of cephalosporin chemistry [123] and involves the sequential sulfoxidation, esterification and dehydratation/expansion of the penicillin nucleus. The yields were low, initially, but improved as the result of a considerable research effort [124]. An optimized

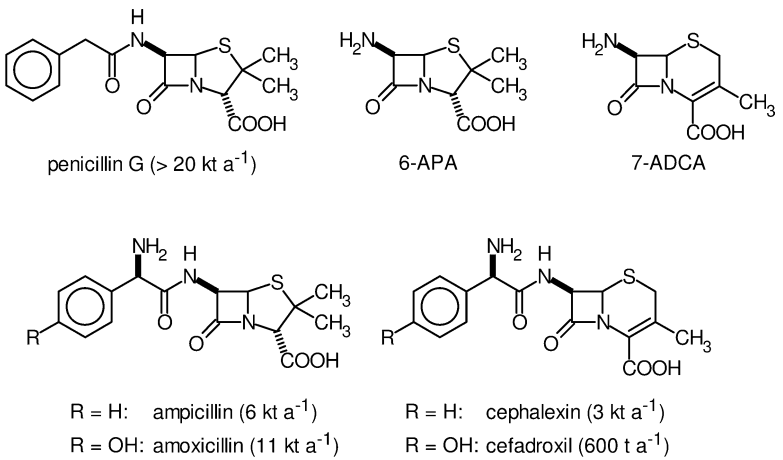


Fig. 8.23 Penicillin G and its progeny.

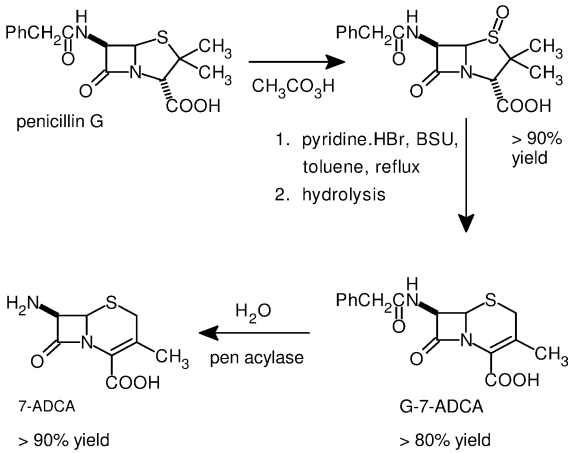


Fig. 8.24 Chemoenzymatic synthesis of 7-ADCA from penicillin G. Abbreviation: BSU, *N,N'*-bis(trimethylsilyl)urea.

procedure (Fig. 8.24) afforded G-7-ADCA in >80% yield [125, 126]; subsequent enzymatic hydrolysis affords 7-ADCA.

The chemical ring expansion process has been employed universally for over 20 years and has remained typical for the times in which it was conceived: although stoichiometrically efficient it depends on hazardous, highly active chemicals, which are difficult to recover and reuse, and it generates an excessive amount of waste.

The relationship between the biosynthesis of the penicillin and cephalosporin nuclei [127] is shown in Fig. 8.25. The common intermediate in the biosynthesis of penicillins and cephalosporins is isopenicillin N (IPN), which in *Penicillium* is converted into penicillin G by replacement of the L-2-aminoadipyl side-chain with externally supplied phenylacetic acid, mediated by IPN acyl transferase (IPN AcT). In the cephalosporin-producing *Acremonium chrysogenum*, IPN is subjected to an enzymatic ring expansion.

The replacement of the traditional, chemoenzymatic process for 7-ADCA by a biotransformation, preferably in the course of the fermentation, was greatly desired [122] but far from trivial to accomplish. It should be noted that the enzymatic ring expansion step (see Fig. 8.25) is not very selective, and the productivity is modest even in natural cephalosporin producing organisms.

Attempts to perform an enzymatic ring expansion on penicillin G were not successful, due to the strict selectivity of desacetoxycephalosporinase (expandase) [127a], even when genetically modified [128]. A solution was found by feeding, instead of phenylacetic acid, adipic acid to a culture of a transgenic *P. chrysogenum* that expressed the gene for expandase, resulting in the formation of adipyl-7-ADCA (Fig. 8.26) [121, 129]. The production level of adipyl-7-ADCA was improved via site-directed mutagenesis of the expandase [130] and by DNA shuffling to relieve suspected kinetic bottlenecks [131].

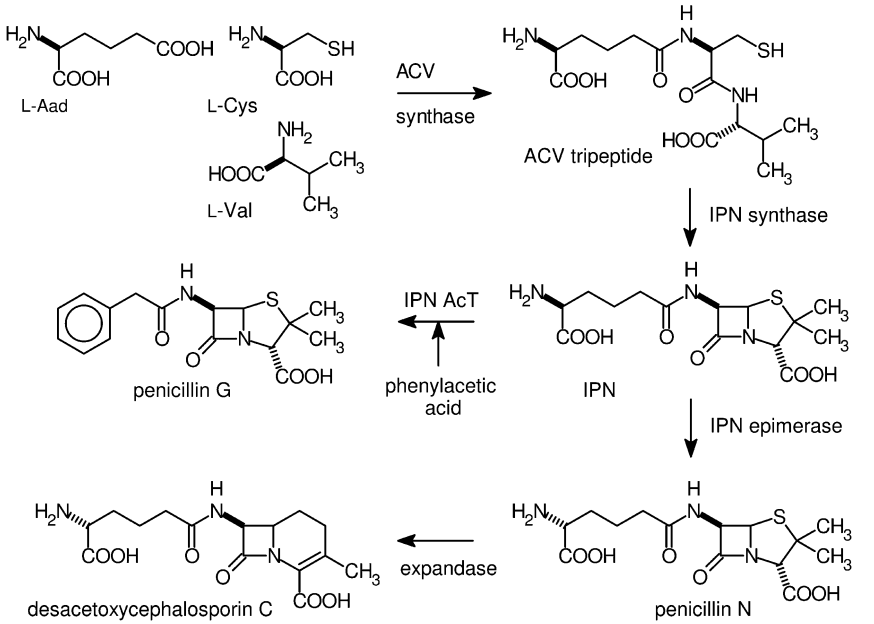


Fig. 8.25 Biosynthesis of the penicillin and cephalosporin nuclei. Compounds: Aad, L-2-aminoadipate; IPN, isopenicillin N. Enzymatic activity: IPNAcT, IPN acyltransferase.

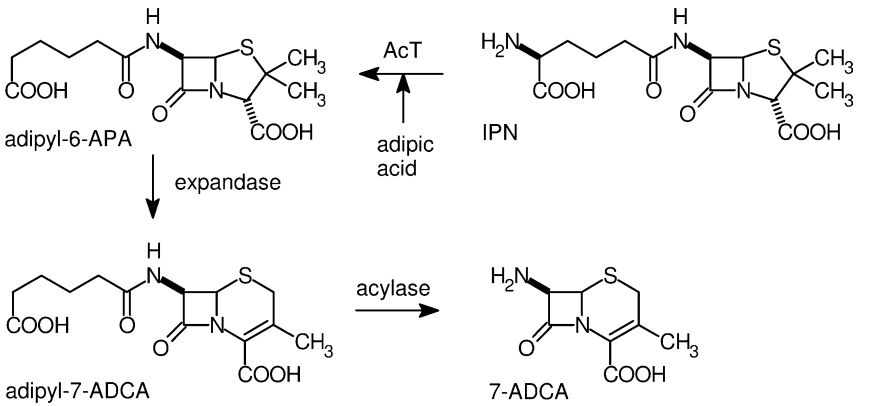


Fig. 8.26 An all-bio process for 7-ADCA: biosynthesis of adipyl-7-ADCA in a recombinant *P. chrysogenum* and enzymatic hydrolysis of the adipyl group.

The enzymatic ring expansion is neither complete nor selective, necessitating product isolation via chromatography in a simulated moving bed system. Because adipyl-7-ADCA rather than G-7-ADCA is produced, a new enzyme, adipyl-7-ADCA acylase, was developed to remove the side chain from adipyl-7-ADCA [132, 133], as the latter is not a substrate for penicillin G acylase.

DSM now produces green 7-ADCA in a new production facility in Delft and has closed down the installations in Delft and Matosinhos (Portugal) that employed the traditional technology. In conclusion, the key β-lactam nucleus 7-ADCA is now synthesized via biotransformations at great savings in chemicals, solvents and energy [134].

8.2.9 Riboflavin

Riboflavin (vitamin B_2) is an essential nutritional factor for humans (0.3–1.8 mg d^{-1}) and animals (1–4 mg (kg diet)$^{-1}$), who need it as a precursor for flavoproteins [135]. It is produced at a volume of approx. 3 kt a^{-1}, mainly as an animal feed additive. Approx. 300 t a^{-1} is used as a food additive and food colorant (E-101) and the remainder (500 t a^{-1}) is used in pharmaceutical applications. Major producers are Roche (Switzerland), BASF (Germany), Archer-Daniels-Midland (USA) and Takeda (Japan). Microbial and chemical production have coexisted for many years but the latter has recently been phased out [136].

For many years, riboflavin has been produced from D-ribose (Rib) and 3,4-xylidine, via the Karrer-Tishler process (see Fig. 8.27) [135], which pushed the existing fermentative procedures from the market in the late 1960s [137], although the laborious synthesis of Rib [135] was a serious drawback. Later, a fermentative process for Rib [138] was adopted. The Karrer-Tishler process is, from the green standpoint, not one of the worst as the overall yield is 60%, relatively little organic solvents are used and few auxiliary groups are discarded.

The biosynthesis of riboflavin, from the nucleotide guanosine triphosphate (GTP), requires at least six enzymatic activities and is subject to a complex regulation architecture [136, 139]. The genes that encode the enzymes have been identified and cloned [136].

The biosynthetic pathway of riboflavin in *B. subtilis* is outlined in Fig. 8.28. The starting compound GTP is, as well as the other purines, tightly regulated as these normally are not present in the cell in appreciable amounts [140]. The final step is a dismutation of DRL into riboflavin and ArP. The six enzymatic activities involved (not counting the phosphatase) are encoded by four genes, which are closely grouped on the *rib* operon [139].

The aerobic microbial production of riboflavin in various microbes dates back to the 1930s [141] but was discontinued in the late 1960s because the chemical process described above was more profitable. Fermentation soon (Merck, 1974) made a comeback, however, and subsequently has steadily eroded the position of the Karrer-Tishler process. Around 1990, production levels of 15–20 g L^{-1} had been achieved [136], up from 2 g L^{-1} in 1940 [141], causing the product to crys-

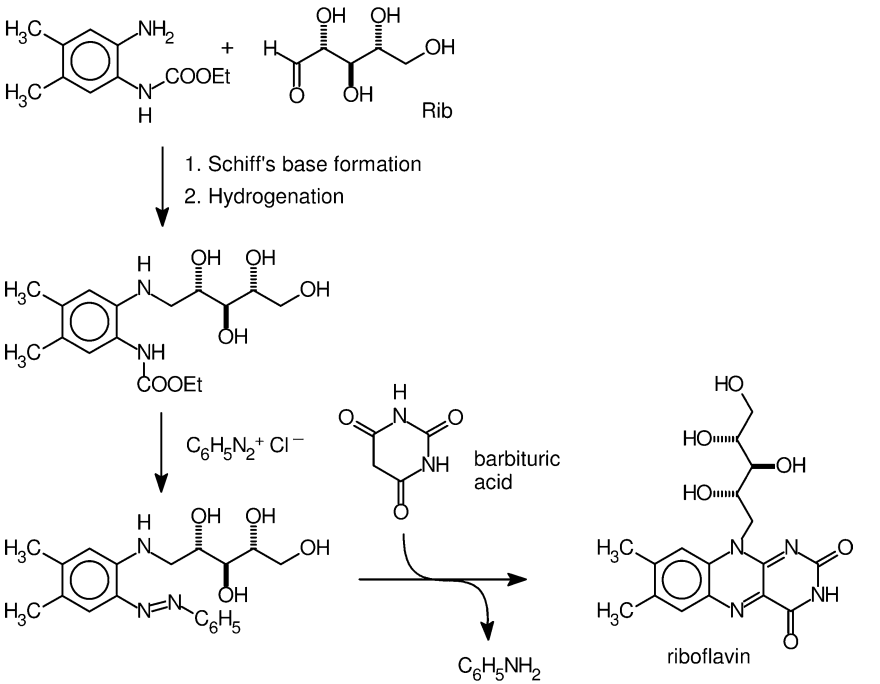


Fig. 8.27 The Karrer-Tishler industrial process for riboflavin. Abbreviation: Rib, D-ribose.

tallize from the fermentation medium. These "natural riboflavin overproducing" organisms mainly resulted from classical strain improvement as metabolic design studies have been initiated only recently [136]. BASF (Germany) started to ferment riboflavin in 1990 and closed down the chemical production in 1996 [136].

Roche (Switzerland), in collaboration with Omnigene (USA), has taken a different approach by engineering a *Bacillus subtilis*, although the latter organism is not a natural overproducer of riboflavin, via a combination of classical mutant selection and fermentation improvement with genetic engineering. The key to success was gene deregulation, replacement of native promoters by constitutive ones and increasing gene copy numbers, based on detailed knowledge of *rib* operon and its control architecture [139, 142].

Downstream processing of riboflavin from fermentation is straightforward, as the product precipitates from the fermentation broth. The crystals are collected by differential centrifuging and a pure product is obtained after repeated crystallization [143]. Roche has replaced its chemical production of riboflavin by the biotechnological process in 2000, with a 50% savings in production costs [54] as well as a 75% reduction in the use of non-renewable materials and very significantly reduced emissions into the environment [144].

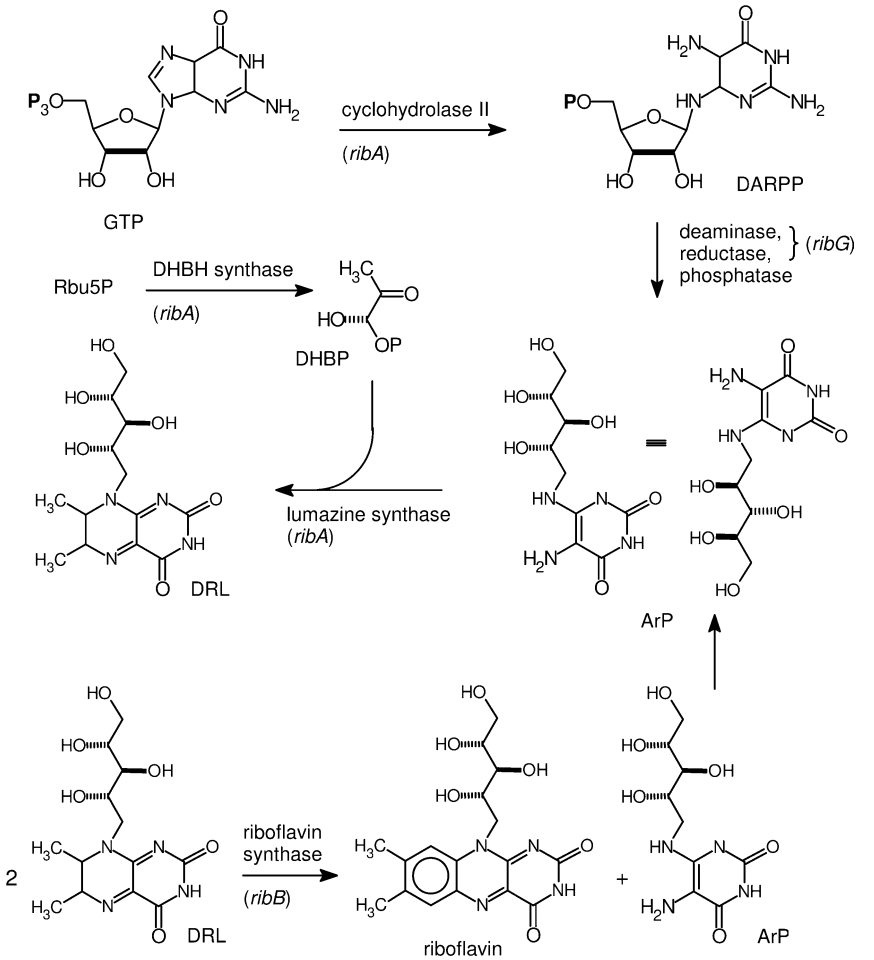


Fig. 8.28 Biosynthesis of riboflavin in bacteria. Compounds: ArP, 5-amino-6-(ribitylamino)-2,4-(1*H*,3*H*)-pyrimidinedione; DARPP, 2,5-diamino-6-ribosylamino)-4(3*H*)-pyrimidinedione-5′-phosphate; DHBP, L-(*S*)-3,4-dihydroxy-2-butanone-4-phosphate; DRL, 6,7-dimethyl-8-ribityllumazine; GTP, guanidine triphosphate; Rbu5P: D-ribulose-5-phosphate.

8.3
Chemical and Chemoenzymatic Transformations of Carbohydrates into Fine Chemicals and Chiral Building Blocks

We have noted in Section 8.2.1 that the structural complexity of carbohydrates is an obstacle to their application as a feedstock for chemistry. Nonetheless, the complexity of carbohydrates can be exploited in either of two ways. The first is to match the starting material to the structure of the product; L-ascorbic acid is the best-known

example of this approach. The other is to break down the carbohydrate into enantiomerically pure C_3 and C_4 units that are broadly applicable building blocks.

8.3.1 Ascorbic Acid

L-Ascorbic acid (vitamin C, ASA) is produced on a scale of 80 kt a^{-1} worldwide; it is used in food supplements, pharmaceutical preparations, cosmetics, as an antioxidant in food processing and a farm animals feed supplement [145]. It is synthesized *in vivo* by plants and many animals, but not by primates, including Man, or microbes.

The industrial production of ASA has been dominated by the Reichstein-Grussner process (Fig. 8.29) [146] since the mid 1930s. Although the intermedi-

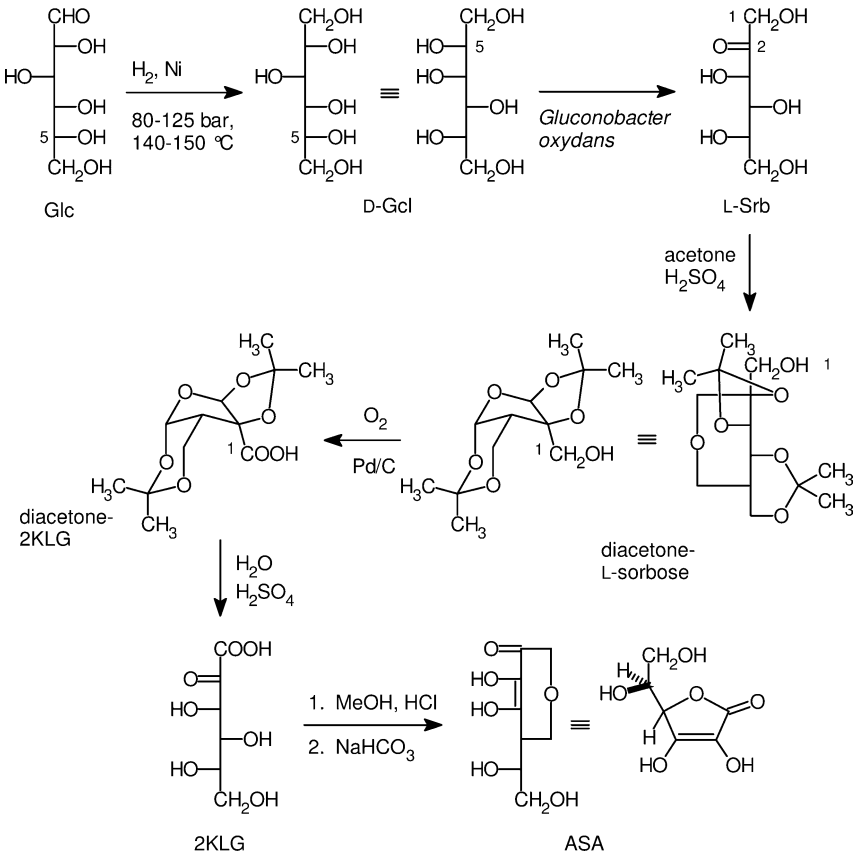


Fig. 8.29 Industrial, chemical synthesis of L-ascorbic acid. Experimental conditions have been taken from [145]. Structures (except those of the acetone derivatives) are in open-chain Fischer projection to make the stereochemical relationships clear (although such structures do not exist in aqueous solution). Compounds: ASA, L-ascorbic acid; Glc, D-glucose; D-Gcl, D-glucitol (sorbitol); 2KLG, 2-keto-L-gulonate; L-Srb, L-sorbose.

ates remained the same, every step in the sequence has been optimized [147] and there is none that has survived unchanged since the days of Reichstein. Biotechnological approaches to ASA have been developed over approx. 20 years [148, 149] and are now being introduced into industrial practice.

ASA is a lactone of 2-keto-L-gulonic acid (2KLG, see Fig. 8.29), which is not readily accessible from a natural precursor. Glc, the preferred starting material, has the correct stereochemistry if its reducing and non-reducing ends are swapped. The solution is catalytic hydrogenation of Glc into D-glucitol (D-Gcl, sorbitol [150]) followed by biooxidation of the hydroxy group that originally had been at C-5 to give L-sorbose (L-Srb). This part of the Reichstein procedure is highly efficient (yield approx. 95%, STY 250 g L^{-1} d^{-1} [151, 152]).

The final oxidation step of the primary alcohol at C-1 in L-Srb requires acetone protection, which is carried out in a standard textbook way in the presence of an excess of sulfuric acid. The oxidation at C-1 has been accomplished in a number of ways [147]; it seems that nowadays aerobic oxidation in the presence of palladium or platinum is preferred. Deprotection, requiring additional sulfuric acid, affords 2KLG, which is transformed into ASA via esterification and lactonization. Alternatively, the diacetone derivative of 2KLG can be converted directly into ASA by treatment with HCl in an organic solvent.

Obvious shortcomings of the Reichstein procedure are the modest yield (50–60% from L-sorbose is often mentioned) and the acetone protection–deprotection sequence. Overall, 1 ton of sodium sulfate is produced per ton of ASA (acetone is recycled). It should be noted, on the other hand, that the catalytic hydrogenation and microbial oxidation steps are in all respects highly efficient, even by today's standards [153] and impeccably green [154].

Grafting a microbial oxidation of L-Srb into 2KLG on the Reichstein process, which is an obvious improvement to the latter, results in a two-stage fermentative process (Fig. 8.30a). China was the first country to develop [155] and universally adopt such a process [156]. Its production costs are said to be 30% lower that those of the traditional process [157]. Cerestar has claimed an 89% yield (100 g L^{-1}; STY 78 g L^{-1} d^{-1}) of 2KLG from L-Srb in a mixed culture of *G. oxydans* and *Bacillus thuringiensis* [151]. Such processes have now been introduced in Europe, for example by KGS (Krefeld, Germany) and DSM (Dalry, Scotland).

A one-stage, mixed-culture fermentation of D-Gcl into 2KLG [149, 158] has been disclosed by Archer-Daniels-Midland (USA) [159] and, presumably, is at the basis of ADM's production process for ASA [54, 160]. 2KLG yields of up to 95% (>100 g L^{-1}; STY 70 g L^{-1} d^{-1}) have been claimed.

A single-microbe process to convert D-Gcl into 2KLG, which would be preferable, faces the recurrent problem that 2KLG, as well as the intermediates, tend to enter the central metabolism. Hence, a major theme in all recent research is to restrict the production of 2KLG, as far as possible, to the periplasm, out of reach from the central metabolism in the cytosol [149].

Initial attempts to convert D-Gcl into 2KLG in one microbe met with low yields and much L-idonate (IDA, see Fig. 8.30a) was produced [161, 162]. The formation of IDA was eliminated [163] and the yield of KLG was increased to

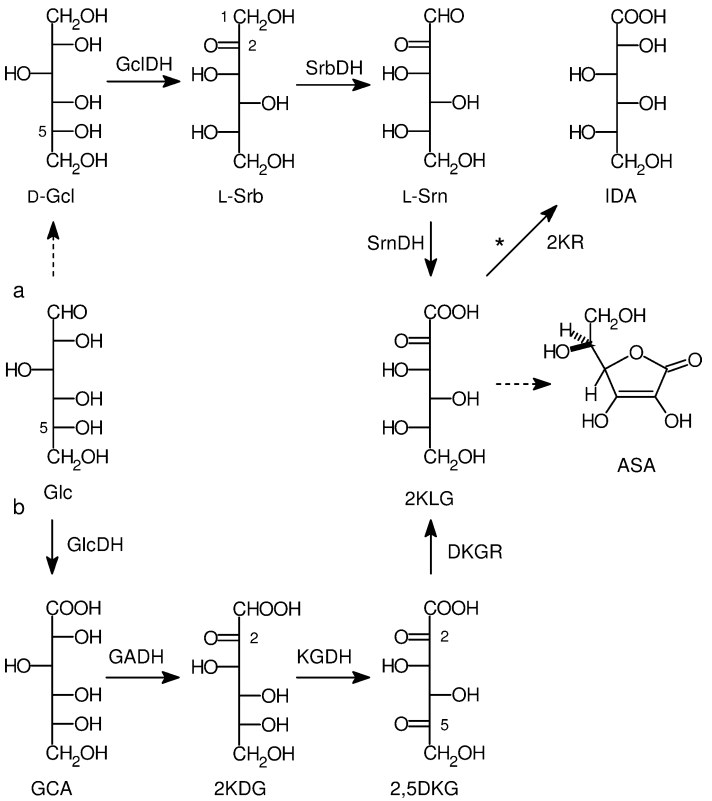


Fig. 8.30 Microbial routes to 2-keto-L-gulonate; (a) via L-sorbose; (b) via 2,5-diketo-L-gulonate; *: unwanted side reaction; non-microbial steps are indicated by hatched arrows. Compounds: 2,5DKG, 2,5-diketo-D-gluconate; D-Gcl, D-glucitol; IDA, L-idonate; 2KDG, 2-keto-D-gluconate; L-Srb, L-sorbose; L-Srn, L-sorbosone. Enzymatic activities: DKGR, 2,5-diketo-D-gluconate reductase; GADH, D-gluconate dehydrogenase; GclDH, sorbitol dehydrogenase; GlcDH, glucose dehydrogenase; KGDH, 2-keto-D-gluconate dehydrogenase; 2KR, 2-ketoaldonate reductase; SrbDH, L-sorbose dehydrogenase; SrnDH, L-sorbosone dehydrogenase.

82% (88 g L^{-1}; 29 g L^{-1} d^{-1}) by appropriate mutagenesis. Although yield and titer are good, the STY is less than half of that of the two-stage fermentation described above [151].

A completely different approach, which is outlined in Fig. 8.30b, involves the production of 2KLG via the D-enantiomer (2KDG), using a redox procedure to invert the stereochemistry on C-5. A two-stage fermentative procedure based on this latter principle was already published in 1982 and involved transformation of Glc into 2,5DKG (yield 95%, 329 g L^{-1}; STY 300 g L^{-1} d^{-1}) followed by a microbial reduction with Glc as the reducing agent [164]. 2KLG was obtained in 93% yield (86% from glucose, 69% when the glucose reductant in the second stage is taken into account).

Combining the required activities in a single organism was more challenging, as explained above. The product yield and titer, which were initially low [165], were improved [149] and recently Genencor has announced the fermentative synthesis of 2KLG in a recombinant *Pantoea citrea* sp. [22], which is the outcome of extensive mutagenesis [166, 167]. A product titer of 120 g L^{-1} [22] and an STY of 62 g L^{-1} d^{-1} [167] have been claimed, but yields on Glc have not been disclosed. The ultimate target set by Genencor is an STY of 240 g L^{-1} d^{-1} [167].

In parallel with the fermentative process, Genencor has developed an *in vitro* process to convert Glc into 2,5DKG [22, 168]. This latter system encompasses a redox couple of free GlcDH and 2,5DKGR that mediate the first and last steps in the route, complemented by GADH and 2KGDH in a permeabilized *P. citrea* (Fig. 8.31). Its virtues are said to be a quantitative yield of 2KLG without byproducts, improved STY and simple DSP [22].

Genencor and Eastman have announced plans for the production of ASA [169], presumably based on either the fermentative or the *in vitro* processes developed by Genencor.

A fermentative or biocatalytic procedure for 2KLG calls for a completely different approach to the DSP of the latter and possibly also for the conversion of 2KLG into ASA. The basic problem is that 2KLG is dissolved in the culture supernatant as a salt, due to the necessity to titrate the culture with base to maintain neutrality. The conversion of 2KLG into ASA, in contrast, requires the free acid. Traditional solutions, such as spray-drying and acidification with sulfuric acid [170] and evaporative crystallization [171], are energy intensive and coproduce an equivalent of salt, which is, parenthetically, one of the objections against the Reichstein process.

A considerable number of recent patent applications describe the new ideas in this field. These are, for example, the salt-free recovery of 2KLG from its sodium salt by electrodialysis [172]; the Na^+ ions are recovered as NaOH and reused as titrant. An integrated procedure, based on reactive chromatography, for the recovery and further reaction of 2KLG has been demonstrated; the yield of 2KLG ester was 93% [173]. Combining reactive chromatography with acid-catalyzed lactonization of 2KLG at 90–110 °C [174] bypasses the esterification step [175]; in an industrial environment SMB chromatography would be preferred [173, 175a]. Such techniques replace rather than eliminate the problem of water removal and would require non-distillative techniques, such as pervaporation, to become really energy-efficient.

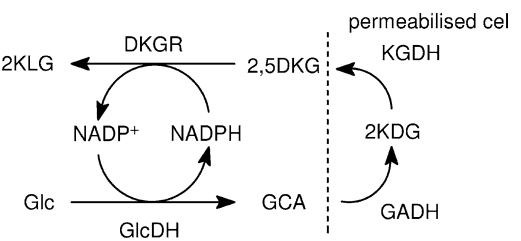


Fig. 8.31 Production of ASA in an *in vitro* multienzyme system. Compounds and enzymatic activities as in Fig. 8.30b; GlcDH, glucose dehydrogenase.

De-novo fermentation of ASA in an acid-tolerant yeast, combined with in-process product removal, has the potential of eliminating salt production altogether. Unfortunately, there is no microbe that naturally produces ASA and the reported yields are minute until now [176].

After many years of seeming stagnation, the prospects are now good that the chemical process for ASA will be swept from the market by greener, more efficient and cheaper to operate procedures based on biotechnology. The ultimate objective, *de-novo* fermentation from Glc, is still elusive but there is little doubt that competition between the producers eventually will cause one to be developed.

8.3.2 Carbohydrate-derived C_3 and C_4 Building Blocks

The optimum size for a generally applicable chiral building block is 3–4 carbon atoms [177]. Hence, efficient procedures for breaking down carbohydrates into such building blocks are a *conditio sine qua non* for their application. An ideal candidate for such a transformation is D-mannitol, which would afford two molecules of an enantiopure C_3 building block upon oxidative cleavage of the bond between carbon atoms 3 and 4. D-Mannitol is produced at an estimated volume of 30–40 kt a^{-1}, mainly via catalytic hydrogenation of Fru or Glc-Fru mixtures [178].

Selectivity, which is always a problem in carbohydrate chemistry, generally requires protection of the hydroxy groups that are to be recovered unchanged. The oxidative cleavage of 1,2:5,6-di-*O*-isopropylidene-D-mannitol (DAM) into two molecules of (*R*)-2,3-*O*-isopropylideneglyceraldehyde (see Fig. 8.32) that results from oxidation with periodate has been known for many years. The green credentials of periodate are poor, however, as it is converted into a stoichiometric amount of iodate, which is difficult to reoxidize and recycle. Catalytic oxidation, which is preferable, has been accomplished with hypochlorite in the presence of ruthenium(III) chloride [179]. With this latter system, DAM was converted into the (*R*)-D-glycerolic acid derivative in 97% yield. As an additional advantage, the acid is much more stable than the aldehyde.

An oxidative route to (*S*)-glycerolic acid would require L-mannitol, which is, however, a rare and expensive compound. L-Ascorbic acid (ASA), in contrast, is a readily available commodity that has been converted into (*S*)-glycerolic acid via acetone protection and a two-step oxidation (Fig. 8.33) [179].

Selective transformation of carbohydrates and protecting group techniques are nearly synonymous. A remarkable exception has recently been demonstrated with the oxidative cleavage of 4-linked hexopyranose sugars, such as maltose, into (*S*)-3-hydroxy-γ-butyrolactone (*S*3HBL) [180]. The reaction sequence (see Fig. 8.34a) involves a base-catalyzed isomerization into the corresponding ketose, elimination at C-4 and oxidative cleavage of the 3,4-diketo moiety. The product was obtained in 85% (mol/mol) yield by column chromatography or distillation. *S*3HBL is a widely applicable synthetic building block [177, 181]. The mass yield

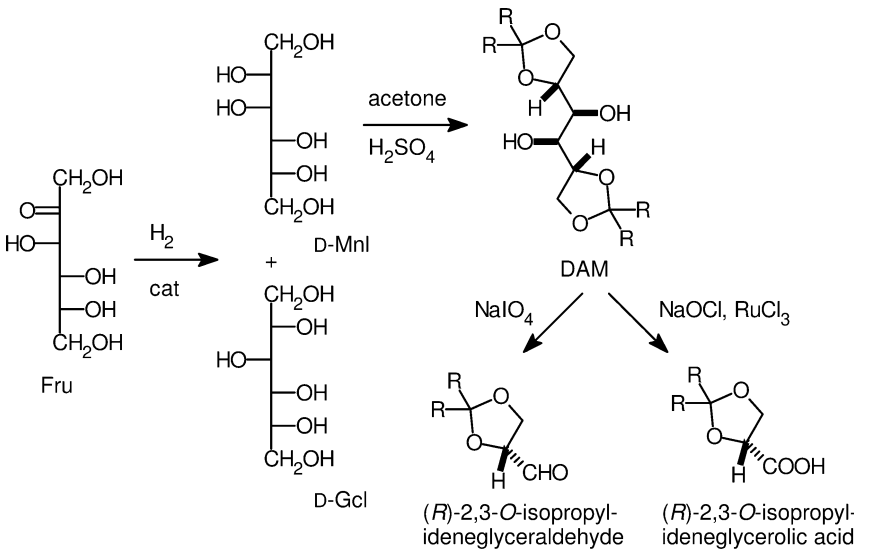


Fig. 8.32 Synthetic routes to enantiomerically pure (*R*)-C_3 building blocks. Compounds: Fru, D-fructose; D-Gcl, D-glucitol; D-Mnl, D-mannitol; DAM, 1,2:5,6-di-*O*-isopropylidene-D-mannitol.

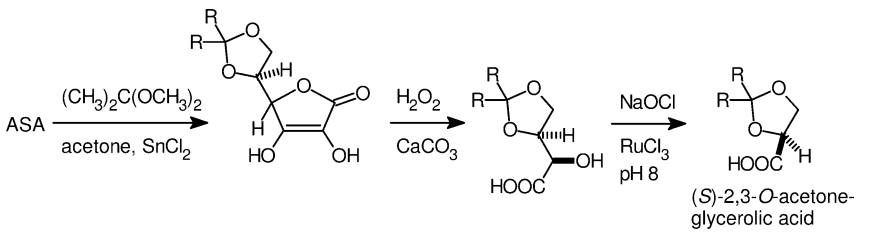


Fig. 8.33 Conversion of L-ascorbic acid into an (*S*)-glycerolic acid derivative. Compound ASA, L-ascorbic acid.

of *S*3HBL from maltose is only 33% maximum (28% in practice), because the non-reducing carbohydrate unit is sacrificed. Much higher yields, up to a theoretical 70% (w/w), can be obtained from polysaccharides, as each elimination step generates a new reducing end but, due to low concentration of the latter, the reaction will be slow. It has been suggested that maltodextrins with dp 10 would strike a good balance between yield and reaction rate [182].

The configuration at C-3 in *S*3HBL is derived from C-5 in the hexose system, which is D in all readily available oligo- and polysaccharides. Hence, the starting material for *R*3HBL is restricted to L-monosaccharides such as L-arabinose, which is available in abundance from sugar beet pulp, but requires a protection/activation strategy at C-4. It was accordingly shown that the readily avail-

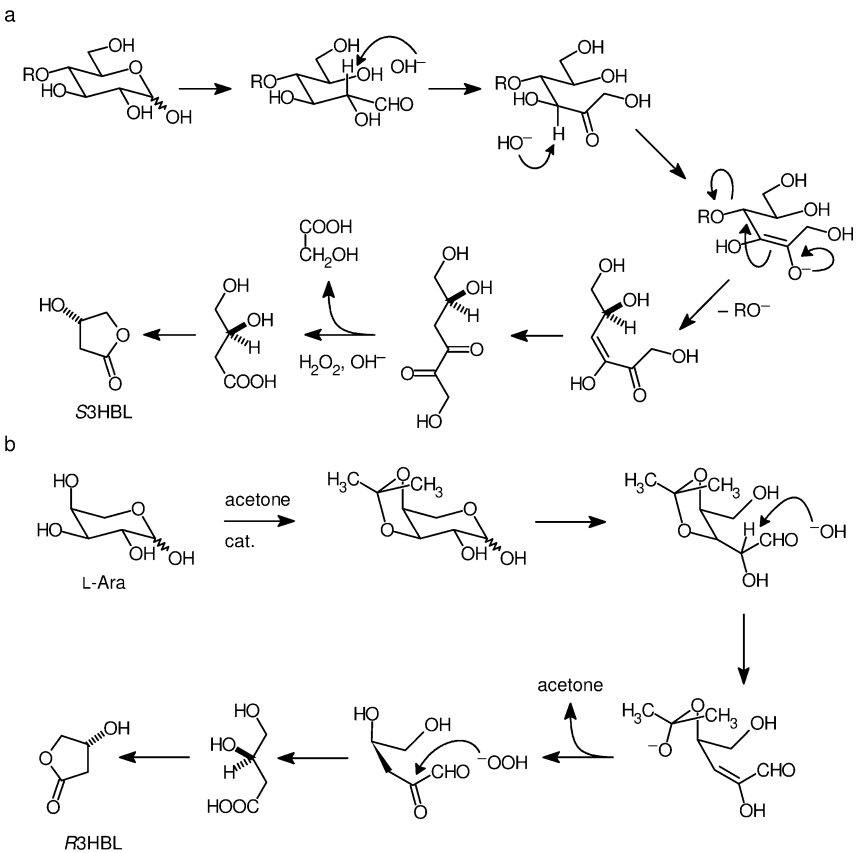


Fig. 8.34 Synthesis of (*R*)- and (*S*)-3-hydroxybutyrolactone from carbohydrate starting materials. Compounds: L-ARA, L-arabinose, *R*3HBL, (*R*)-3-hydroxybutyrolactone, *S*3HBL, (*S*)-3-hydroxybutyrolactone.

able derivative 3,4-*O*-isopropylidene-L-arabinose could be converted, via a similar sequence as described above (Fig. 8.34 b), into *R*3HBL in 96% yield [183].

8.3.3
5-Hydroxymethylfurfural and Levulinic Acid

It has often been proclaimed that 5-hydroxymethylfurfural (HMF, Fig. 8.35) could be an ideal cross-over compound between carbohydrates and petrochemistry [184], as it is a bifunctional heteroaromatic compound that is accessible from fructose in one step. It was expected that HMF could be developed into a valuable synthetic building block and that its derivatives, such as furan-2,5-dicarboxylic acid (FDA), would be able to compete with fossil-derived monomers for use in thermostable polyesters and polyamides.

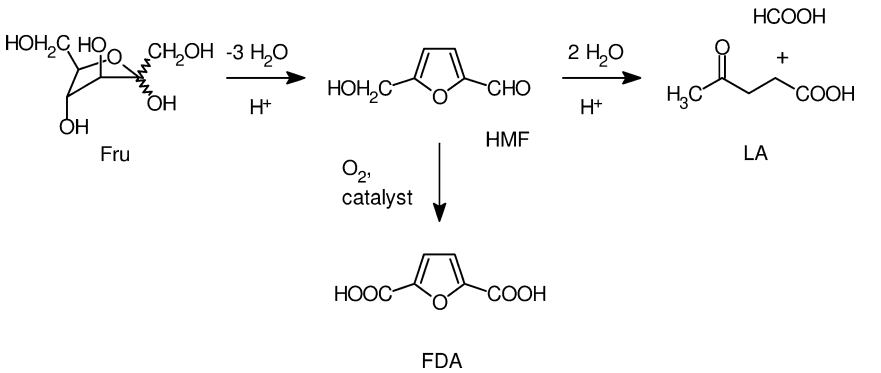


Fig. 8.35 Transformation of D-fructose into 5-hydroxymethylfurfural and furan-2,5-dicarboxylic acid. Compounds: Fru, D-fructose; HMF, 5-hydroxymethylfurfural; FDA, furan-2,5-dicarboxylic acid, LA, levulinic acid.

Fructose, or a fructose-containing polysaccharide, can be transformed into HMF by aqueous sulfuric acid at 150 °C via a number of isomerization and dehydration steps [185]. Presumably, such a procedure was at the basis of the pilot-scale production of HMF by Südzucker (Germany) [186]. Major problems are the competing rehydration of HMF into levulinic acid (4-oxopentanoic acid, LA, see Fig. 8.35) and the formation of polymeric side-products, which necessitate chromatographic purification. Yields have stayed modest (approx. 60%), in consequence.

Various strategies, such as the use of solid acids, reaction in biphasic media or in anhydrous DMSO, have been attempted to improve the yield and selectivity [187]. Recently, HMF has been prepared in 1,2-dimethoxyethane, combined with water removal [188], in ionic liquid media [189] and in supercritical acetone [190] but the selectivity for HMF could not be improved beyond approx. 80%, which is undesirably low.

In conclusion, the selective conversion of biomass into HMF is still a formidable obstacle after 50 years of study. Due to the modest selectivity, the DSP is too elaborate for a commodity chemical and the target price of \$ 2000 t^{-1} [190] is still elusive.

The interest in FDA arises from its possible application as a renewable-derived replacement for terephthalic acid in the manufacture of polyesters. A multitude of oxidation techniques has been applied to the conversion of HMF into FDA but, on account of the green aspect, platinum-catalyzed aerobic oxidation (see Fig. 8.35), which is fast and quantitative [191], is to be preferred over all other options. The deactivation of the platinum catalyst by oxygen, which is a major obstacle in large-scale applications, has been remedied by using a mixed catalyst, such as platinum–lead [192]. Integration of the latter reaction with fructose dehydration would seem attractive in view of the very limited stability of HMF, but has not yet resulted in an improved overall yield [193].

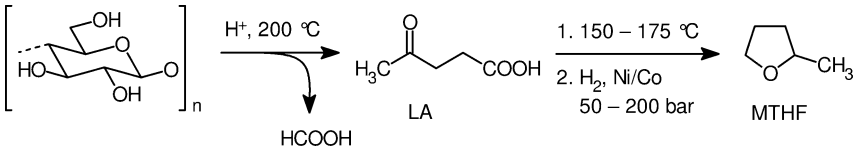


Fig. 8.36 Production of levulinic acid and transformation into 2-methyltetrahydrofuran. Compounds: LA, levulinic acid; MTHF, 2-methyltetrahydrofuran.

We note that, even if the price of HMF would drop to \$ 2000 t^{-1}, FDA derived from the latter would be too expensive by a factor of eight (at least) to compete with terephthalic acid at \$ 400 t^{-1}. Hence, there is little prospect that such a substitution will be realized in practice.

LA, which has already been mentioned as an undesirable side-product in the synthesis of HMF, is also a fine chemical, with a wide range of small-scale applications, that has been produced since 1870 [194].

The possible development of LA into a cross-over chemical between carbohydrates and petrochemicals has recently spurred the development of more efficient procedures for its production. Most start from cheap starting materials such as lignocellulose residues and waste paper in acidic medium at approx. 200 °C; the theoretical yield of such a procedure is 0.71 kg kg^{-1} (see Fig. 8.36). In a patent application for a two-stage procedure the claimed yields were 62–87% of the theory, depending on the raw material [195]. A much simpler, extrusion-based procedure has been described but even when fitted with a second stage the yield was not better than 66% of the theory [196]. Efficient DSP is not trivial and the chromatographic separation that has been described [197] is obviously not compatible with the aimed-for commodity status of LA. Alternatively, the LA can be esterified *in situ* [197].

One potential very large scale application of LA is its transformation, via ring closure and hydrogenation, into 2-methyltetrahydrofuran [197], a possible motor fuel additive. One of the companies involved, Biofine Inc. (now BioMetics) has received the 1999 Presidential Green Chemistry Award for its work on LA.

8.4 Fats and Oils

Naturally occurring oils and fats constitute another important source of renewable raw materials [198]. Whether they are referred to as fats or oils depends on whether they are solid or liquid at room temperature, respectively. They are composed primarily of triglycerides (triesters of glycerol) together with small amounts of free fatty acids, phospholipids, sterols, terpenes, waxes and vitamins. Oils and fats are either of vegetable or animal origin and are produced in the approximate proportions: 55% vegetable oils, 40% land-animal fats and 5% marine oils [199].

8.4.1 **Biodiesel**

Considerable attention is currently being focused on the use of renewable vegetable oils as feedstocks for the production of biodiesel. The latter has obvious benefits in the context of green chemistry and sustainability: (i) since it is plant-derived its use as a fuel is CO_2-neutral, (ii) it is readily biodegradable, (iii) its use results in reduced emissions of CO, SO_x, soot and particulate matter.

Vegetable oils can be used directly, as the triglycerides, to replace conventional diesel fuel but their much higher viscosities make their use rather impractical. Consequently, biodiesel generally consists of a mixture of fatty acid methyl esters (FAMEs), produced by transesterification of triglycerides with methanol (Fig. 8.37). It is worth noting, however, that fatty acid ethyl esters, produced from triglycerides and bioethanol, would constitute a truly green fuel.

The reaction is catalyzed by a variety of both acids and bases but simple bases such as NaOH and KOH are generally used for the industrial production of biodiesel [200, 201]. The vegetable oil feedstock, usually soybean or rapeseed oil, needs to be free of water (< 0.05%) and fatty acids (< 0.5%) in order to avoid catalyst consumption. This presents a possible opportunity for the application of enzymatic transesterification. For example, lipases such as *Candida antarctica* B lipase have been shown to be effective catalysts for the methanolysis of triglycerides. When the immobilized form, Novozyme 435, was used it could be recycled 50 times without loss of activity [201, 202]. The presence of free fatty acids in the triglyceride did not affect the enzymes' performance. The methanolysis of triglycerides catalyzed by Novozyme 435 has also been successfully performed in $scCO_2$ as solvent [203].

Alkali-catalyzed transesterifications have several drawbacks in addition to the problem of free fatty acids and water in the feedstock. They are energy intensive, recovery of the glycerol is difficult, the basic catalyst has to be removed from the product and the alkaline waste water requires treatment. These disadvantages could be circumvented by employing a lipase catalyst. But, in order to be economically viable, the enzyme costs have to be minimized through effective immobilization and recycling.

A spin-off effect of the recent enormous increase in biodiesel production is that the coproduct, glycerol, has become a low-priced commodity chemical. Consequently, there is currently considerable interest in finding new applications of glycerol [204]. One possibility is to use glycerol as the feedstock for fermentative production of 1,3-propanediol (see earlier).

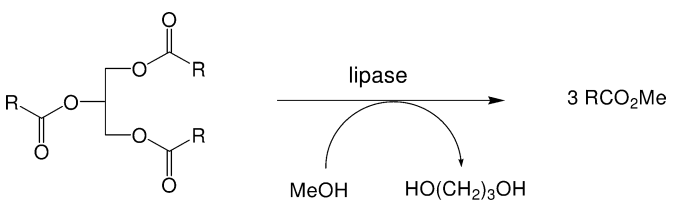


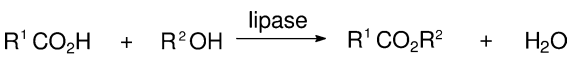
Fig. 8.37 Biodiesel production by transesterification of a triglyceride.

8.4.2
Fatty Acid Esters

Esters of fatty acids with monohydric alcohols find applications as emollients in cosmetics. They are prepared by acid- or base-catalyzed (trans)esterifications [200, 205]. As with biodiesel production, the use of enzymatic catalysis offers potential benefits but in the case of these specialty fatty acid esters there is a special advantage: the products can be labelled as 'natural'. Consequently, they command a higher price in personal care products where 'natural' is an important customer-perceived advantage. Examples include the synthesis of isopropylmyristate by CaLB-catalyzed esterification [206] and n-hexyl laurate by *Rhizomucor miehei* lipase (Lipozyme IM-77)-catalyzed esterification [207] (see Fig. 8.38).

Partial fatty acid esters of polyhydric alcohols such as glycerol, sorbitol and mono- and disaccharides constitute a group of nonionic surfactants and emulsifying agents with broad applications in pharmaceuticals, food and cosmetics. Traditionally, such materials have been prepared using mineral acids or alkali metal hydroxides as catalysts but more recently emphasis has been placed on recyclable solid acids and bases as greener alternatives [200, 205, 207]. For example, sucrose fatty acid esters are biodegradable, nontoxic, nonirritating materials that are applied as 'natural' surfactants and emulsifiers in food and personal care products. They are currently produced by base-catalyzed transesterification but lipase-catalyzed acylations are potentially greener alternatives [208, 209]. Base-catalyzed acylations require high temperatures and produce a complex mixture of mono-, di- and polyesters together with colored byproducts. In contrast, enzymatic acylation can be performed under mild conditions and affords only a mixture of two mono-esters [208, 209].

The major problem associated with the enzymatic acylation of sucrose is the incompatibility of the two reactants: sucrose and a fatty acid ester. Sucrose is hydrophilic and readily soluble in water or polar aprotic solvents such as pyridine and dimethylformamide. The former is not a feasible solvent for (trans)esterifications, for obvious thermodynamic reasons, and the latter are not suitable for the manufacture of food-grade products. The selective acylation of sucrose, as a suspension in refluxing *tert*-butanol, catalyzed by *C. antarctica* lipase B, afforded a 1:1 mixture of the 6 and 6′ sucrose monoesters (Fig. 8.39) [208]. Unfortunately, the rate was too low (35% conversion in 7 days) to be commercially useful.



R^1 = n-$C_{14}H_{29}$; n-$C_{16}H_{33}$

R^2 = i-C_3H_7 ; $CH_3(CH_2)_3CH(C_2H_5)CH_2$—

Fig. 8.38 Lipase-catalyzed synthesis of fatty acid esters.

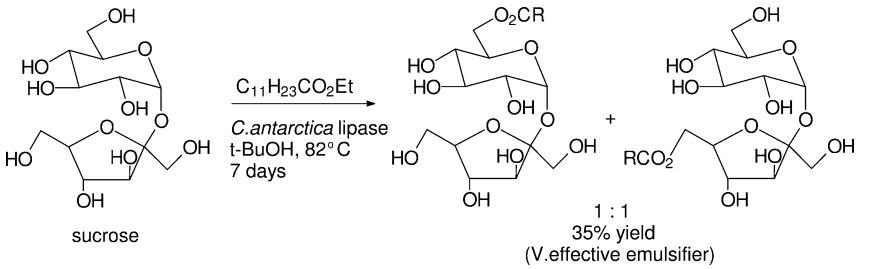


Fig. 8.39 CaLB-catalyzed acylation of sucrose.

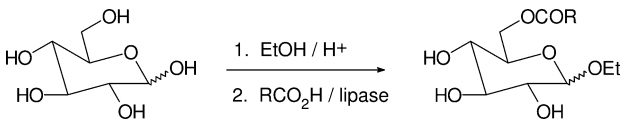


Fig. 8.40 Synthesis of alkylpolyglycosides and their fatty acid esters.

A possible solution to this problem of low rates is to use ionic liquids as solvents. Certain ionic liquids, e.g. those containing the dicyanamide anion, $(NC)_2N^-$, have been shown to dissolve sucrose in concentrations of several hundred grams per litre [210]. The lipase-catalyzed acylation of glucose with fatty acid esters has been shown to occur in a mixture of an ionic liquid, [bmim][BF_4], and *tert*-butanol [211].

Another interesting application involving both carbohydrate and oleochemical feedstocks is the production of alkyl polyglycosides (APGs), for use as biodegradable nonionic surfactants in laundry detergents, by acid-catalyzed reaction of a sugar, e.g. glucose, xylose, lactose, etc., with a fatty alcohol. A greener alternative to conventional mineral acids involves the use of solid acids, such as sulfonic acid resins, clays, zeolites and mesoporous aluminosilicates, as catalysts for glycosidation [212]. Further modification by lipase-catalyzed acylation with fatty acid esters affords surfactants with specialty applications, e.g. in cosmetics (Fig. 8.40).

8.5 Terpenes

A wide variety of terpenes are renewable, sustainable feedstocks for the fine chemical industry [213–215]. For example, they are an important source of ingredients and intermediates for flavors and fragrances and vitamins A and E. The major bulk terpenes are shown in Fig. 8.41.

World production of turpentine oils was ca. 330 000 tons in 1995 [213]. Its major constituents α- and β-pinene are obtained in a pure state by fractional distillation. The flavor and fragrance industry consumes around 30 000 t a^{-1} of pinenes [213].

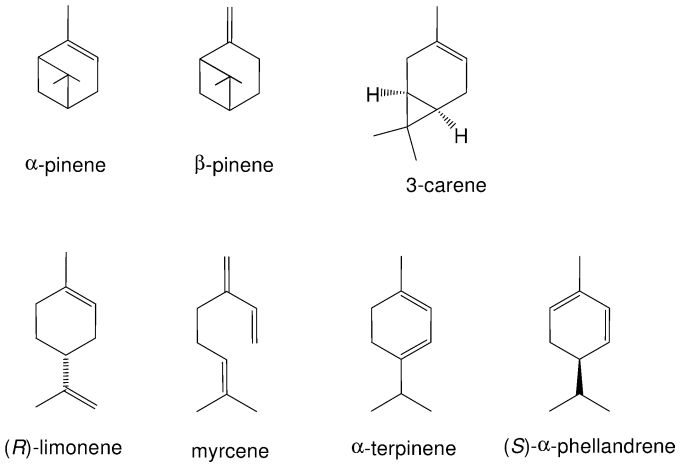


Fig. 8.41 Major terpene feedstocks.

Another major feedstock, limonene, is derived from citrus oils, e.g. as a byproduct of orange juice production, to the extent of around 30 000 t a^{-1} [213].

Catalytic transformations of terpenes are well documented [213–215], comprising a wide variety of reactions: hydrogenation, dehydrogenation, oxidation, hydroformylation, carbonylation, hydration, isomerization and rearrangement, and cyclization.

For example, hydrogenation of *α*- and/or *β*-pinene, over nickel or palladium catalysts, affords *cis*-pinane. Autoxidation of *cis*-pinane (in the absence of a catalyst) gives the tertiary hydroperoxide (see Fig. 8.42) which is hydrogenated to the corresponding alcohol. Thermal rearrangement of the latter affords the important flavor and fragrance and vitamin A intermediate, linalool (Fig. 8.42) [213].

Thermal rearrangement of *β*-pinene affords myrcene (Fig. 8.43) which is the raw material for a variety of flavor and fragrance compounds, e.g. the Takasago process for the production of optically pure L-menthol (see Chapter 1). Dehydro-

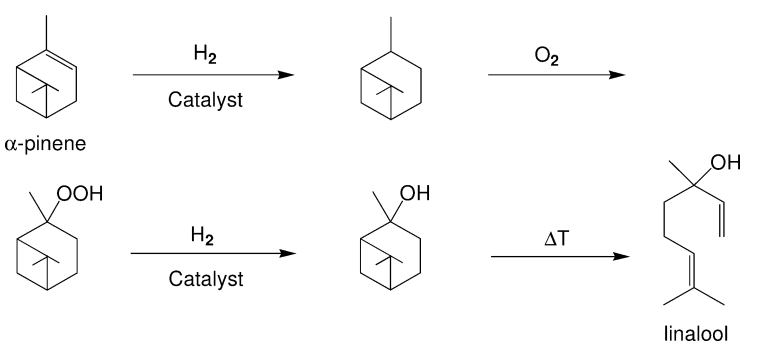


Fig. 8.42 Conversion of *α*-pinene to linalool

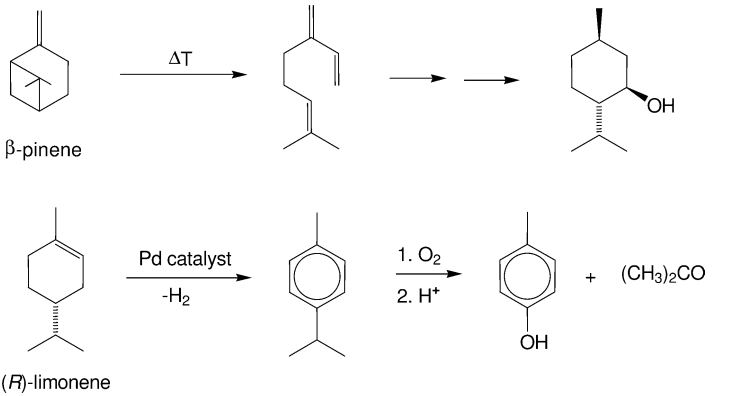


Fig. 8.43 Myrcene and *p*-cymene as key intermediates.

genation of limonene over a palladium catalyst affords *p*-cymene (Fig. 8.43) [213] which is the raw material for *p*-cresol production, via autoxidation and rearrangement, analogous to phenol production from cumene.

Terpenes can also be converted to the corresponding epoxides by reaction with hydrogen peroxide in the presence of a variety of catalysts based on e.g. tungstate, manganese and ruthenium (see Chapter 4 for a discussion of metal-catalyzed epoxidations) [213]. Lewis acid-catalyzed rearrangements of terpene epoxides lead to valuable flavor and fragrance intermediates. Such reactions were traditionally performed with e.g. $ZnCl_2$ but more recently solid Lewis acids such as Ti-beta have been shown to be excellent catalysts for these reactions (see also Chapter 2) [215]. For example, α-pinene can be converted to the corresponding epoxide which undergoes rearrangement in the presence of a Ti-beta catalyst to give the flavor and fragrance intermediate, campholenic aldehyde (Fig. 8.44) [216]. Carvone which has applications in the flavor and fragrance industry and

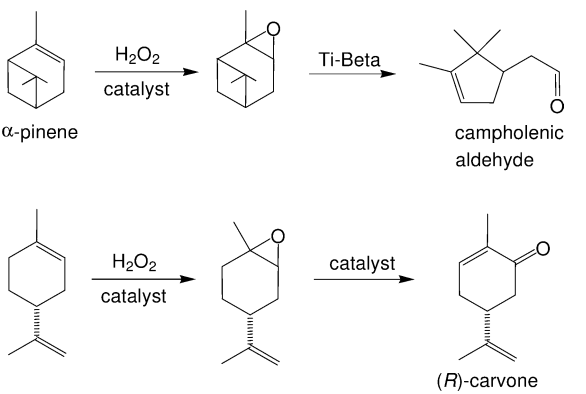


Fig. 8.44 Synthesis of campholenic aldehyde and carvone.

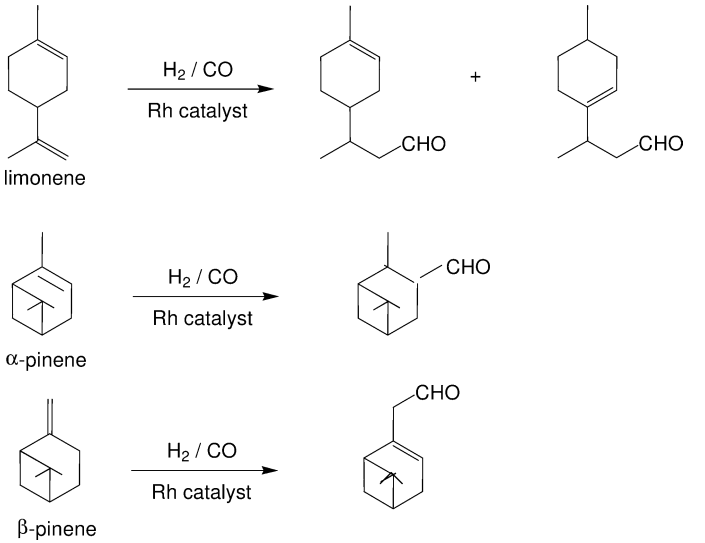


Fig. 8.45 Hydroformylation of terpenes.

as a potato sprouting inhibitor and antimicrobial agent [217], can similarly be prepared by rearrangement of limonene epoxide (Fig. 8.44) [215].

Hydroformylation of terpenes, catalyzed by rhodium complexes (see Chapter 5), is an important route to aldehydes of interest in the perfume industry [215]. Some examples are shown in Fig. 8.45.

Various condensation reactions of terpene-derived aldehydes and ketones, catalyzed by solid bases such as hydrotalcites [215–217], have been described in Chapter 3.

8.6 Renewable Raw Materials as Catalysts

Renewable resources can not only be used as raw materials for green catalytic processes in the chemical industry, but also can be employed as a source of the catalysts themselves. This is surely the ultimate in sustainability. For example, a new solid acid catalyst, for producing biodiesel, has been prepared from sulfonated burnt sugar [218]. The solid, recyclable catalyst is stable at temperatures up to 180 °C and is up to eight times more active than conventional catalysts such as Nafion (see Chapter 2). A carbohydrate, such as sucrose, starch or cellulose, is carbonized to produce polycyclic aromatic sheets which are subsequently treated with sulfuric acid to generate surface sulfonic acid groups. The resulting black amorphous powder can be processed into hard pellets or thin films for use in large-scale biodiesel production.

Another recent innovation is the use of expanded starch as a novel catalyst support [219, 220]. For example, a highly active supported palladium catalyst,

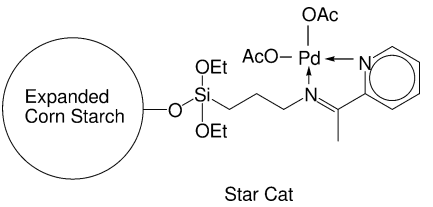


Fig. 8.46 Expanded starch-supported palladium catalyst.

for Heck, Suzuki and Sonagashira reactions, was produced by surface modification of expanded starch by sequential treatment with 3-aminopropyltriethoxysilane and 2-acetylpyridine and palladium acetate (Fig. 8.46). The resulting material was shown to be an effective recyclable catalyst for palladium-mediated C–C bond-forming reactions.

8.7
Green Polymers from Renewable Raw Materials

Renewable feedstocks can also be used as the raw materials for the synthesis of green, biodegradable polymers. A pertinent example is polylactate, derived from lactic acid which is produced by fermentation (see earlier). Another recent example is the production of polycarbonates by reaction of CO_2 with (*R*)-limonene oxide in the presence of a zinc catalyst (Fig. 8.47) [221].

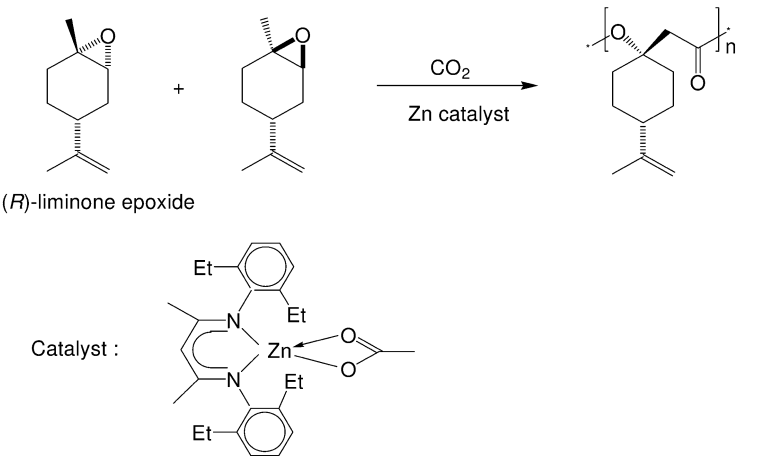


Fig. 8.47 Polycarbonate production from (*R*)-limonene epoxide.

8.8 Concluding Remarks

The transition from a traditional fossil fuel based economy to a sustainable bio-based economy which utilizes renewable raw materials is surely one of the major scientific and technological challenges of the 21st century. It encompasses both the production of liquid fuels, e.g. bioethanol and biodiesel, bulk chemicals, polymers and specialty chemicals. Here again, the key to sustainability will be the development of economically viable, green processes involving the total spectrum of catalysis: heterogeneous, homogeneous and biocatalysis.

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9
Process Integration and Cascade Catalysis

9.1
Introduction

As noted in Chapter 1, the key to success in developing green, sustainable processes is the effective integration of catalytic technologies – heterogeneous, homogeneous, and enzymatic – in organic synthesis. It is also necessary to look at the whole picture; not only the reaction steps but also the downstream processing. This necessitates the integration of a separation step for removal of the product and recovery and recycling of the catalyst into the overall scheme.

Elegant examples of multistep processes involving a series of catalytic steps are the Rhodia process for the manufacture of vanillin and the Lonza process for nicotinamide, discussed in Chapter 1. In these processes the product of each reaction is isolated, and perhaps purified, before going on to the next step. However, the ultimate in integration is to combine several catalytic steps into a one-pot, multistep catalytic cascade process [1–5]. This is truly emulating Nature, where metabolic pathways conducted in living cells involve an elegant orchestration of a series of biocatalytic steps into an exquisite multi-catalyst cascade, without the need for separation of intermediates. Such 'telescoping' of multistep syntheses into a one-pot catalytic cascade has several advantages (see Table 9.1).

They involve fewer unit operations, less solvent and reactor volume, shorter cycle times, higher volumetric and space–time yields and less waste (lower E

Table 9.1 Advantages and limitations of catalytic cascade processes.

Advantages	Limitations
Fewer unit operations	Catalysts incompatible
Drive equilibria in the desired direction	Rates very different
Less solvent/reactor volume	Difficult to find optimum pH, temperature, solvent, etc.
Shorter cycle times	
Higher volumetric and space–time yields	Catalyst recycle complicated
Less waste/lower E factor	Complicated reaction mixtures and work-up

factor). This translates to substantial economic and environmental benefits. Furthermore, coupling of reactions can be used to drive equilibria towards product, thus avoiding the need for excess reagents. On the other hand, there are several problems associated with the construction of catalytic cascades: catalysts are often incompatible with each other (e.g. an enzyme and a metal catalyst), rates are very different, and it is difficult to find optimum conditions of pH, temperature, solvent, etc. Catalyst recovery and recycle is complicated and downstream processing is difficult. Nature solves this problem by compartmentalization of the various biocatalysts. Hence, compartmentalization via immobilization is conceivably a way of solving these problems in cascade processes. It is also worth noting that biocatalytic processes generally proceed under roughly the same conditions – in water at around ambient temperature and pressure – which facilitates the cascading process.

9.2
Dynamic Kinetic Resolutions by Enzymes Coupled with Metal Catalysts

An excellent example of the successful combination of chemo- and biocatalysis in a two-step cascade process is provided by the dynamic kinetic resolutions (DKR) of chiral alcohols and amines. We first suggested [6], in 1993, that (de)-hydrogenation catalysts should be capable of catalyzing the racemization of chiral alcohols and amines via a dehydrogenation/hydrogenation mechanism as shown in Fig. 9.1.

Subsequently the groups of Williams [7] and Backvall [8] showed, in 1996 and 1997, respectively, that lipase-catalyzed transesterification of alcohols could be combined with transition metal-catalyzed racemization to produce an efficient dynamic kinetic resolution of chiral secondary alcohols (Fig. 9.2).

In the system described by Williams a rhodium catalyst was used, in the presence of an inorganic base and phenanthroline, and one equivalent of acetophenone, for the racemization, and a *Pseudomonas sp.* lipase for the acylation using vinyl acetate as the acyl donor (Fig. 9.3).

Backvall, in contrast, used the ruthenium complex **1**, which does not require the addition of an external base, in combination with *p*-chlorophenyl acetate as acyl donor (Fig. 9.4). The latter was chosen because it generates *p*-chlorophenol which does not react with the Ru catalyst. In contrast, vinyl acetate and analo-

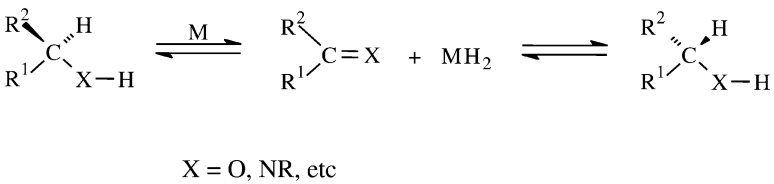


Fig. 9.1 Metal-catalyzed racemization of alcohols and amines.

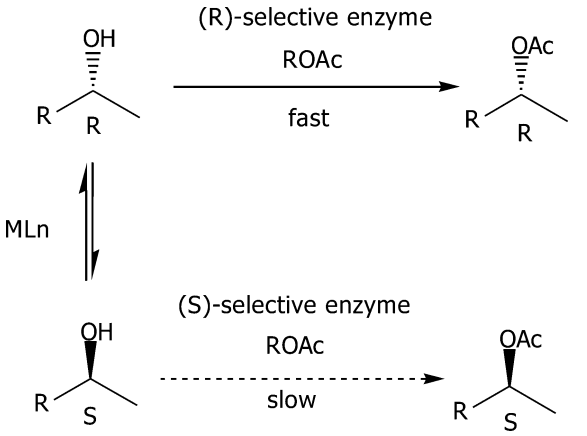


Fig. 9.2 Dynamic kinetic resolution of secondary alcohols.

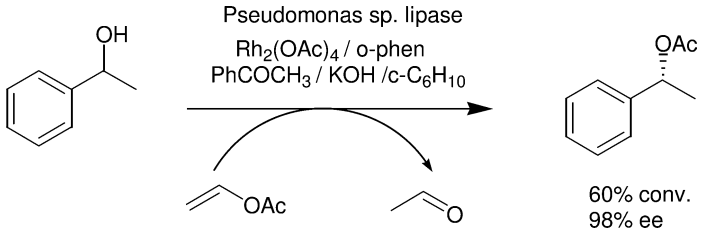
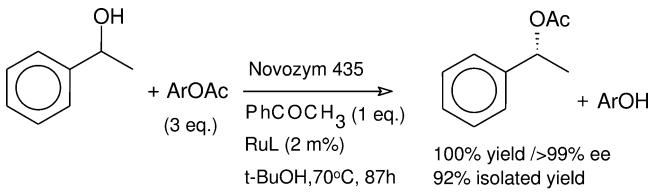


Fig. 9.3 Rh-catalyzed DKR of 1-phenylethanol.



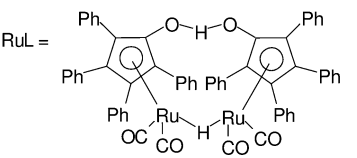


Fig. 9.4 Ru-catalyzed DKR of 1-phenylethanol.

gous acyl donors generate carbonyl compounds as the coproducts that compete for the catalyst. The need for up to one equivalent of additional ketone in these systems is to increase the overall reaction rate. This rate acceleration is a result of increasing the rate of the rate-limiting re-addition of the ruthenium hydride species to the ketone, owing to the higher concentration of ketone. Additional practical disadvantages of the ruthenium system are the need for 3 equivalents of acyl donor, which generate an extra 2 equivalents of *p*-chlorophenol on hydrolytic work-up.

Following on from this initial publication of Backvall, many groups have reported on a variety of ruthenium-based systems for the DKR of secondary alcohols [9–17] mainly with the goal of eliminating the need for added base and ketone and reducing the reaction time by increasing the rate of racemization. Some examples of ruthenium complexes (**1–8**) which have been used as the racemization catalysts in these systems are depicted in Fig. 9.5.

Some of these catalyze the smooth racemization of chiral secondary alcohols at room temperature. However, a major problem which needed to be solved in order to design an effective combination of ruthenium catalyst and lipase in a DKR of secondary alcohols was the incompatibility of many of the ruthenium catalysts and additives, such as inorganic bases, with the enzyme and the acyl donor. For example, the ruthenium catalyst may be susceptible to deactivation by the acetic acid generated from the acyl donor when it is vinyl acetate. Alternatively, any added base in the racemization system can catalyze a competing selective transesterification of the alcohol, resulting in a decrease in enantioselectivity. Consequently, considerable optimization of reaction protocols and conditions was necessary in order to achieve an effective DKR of secondary alcohols.

Several groups have reported [9] ruthenium-based systems that are compatible with the enzyme and acyl donor. The most active of these is the one based on catalyst **4** developed by Bäckvall [17, 18] which effects the DKR of secondary al-

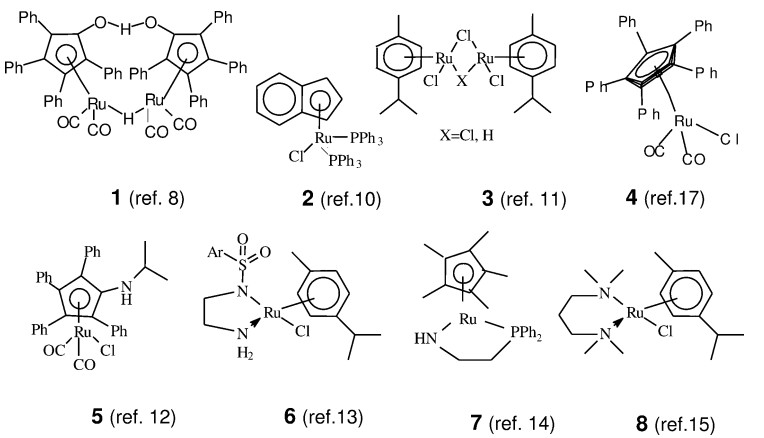


Fig. 9.5 Ruthenium complexes used as alcohol racemization catalysts.

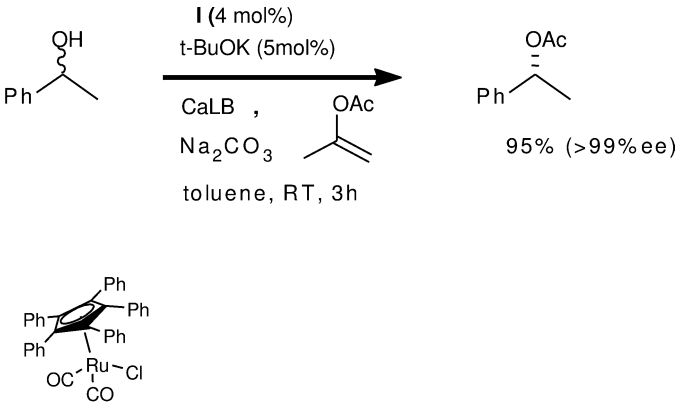


Fig. 9.6 Ru-catalyzed DKR at room temperature.

cohols in 3 h at room temperature, affording excellent enantioselectivities with a broad range of substrates (Fig. 9.6).

An important advance was made with the realization [18] that the function of the base, e.g. potassium *tert*-butoxide, was to convert the catalyst precursor to the active catalyst by displacing the chloride ion attached to ruthenium (Fig. 9.7). Consequently, only one equivalent of base with respect to the Ru catalyst is needed and superior selectivities are observed by performing a separate catalyst activation step, thereby largely avoiding competing base-catalyzed transesterification. The *tert*-butoxide ligand is then replaced by the alkoxide derived from the substrate which then undergoes racemization via a dehydrogenation/hydrogenation cycle as shown in Fig. 9.7.

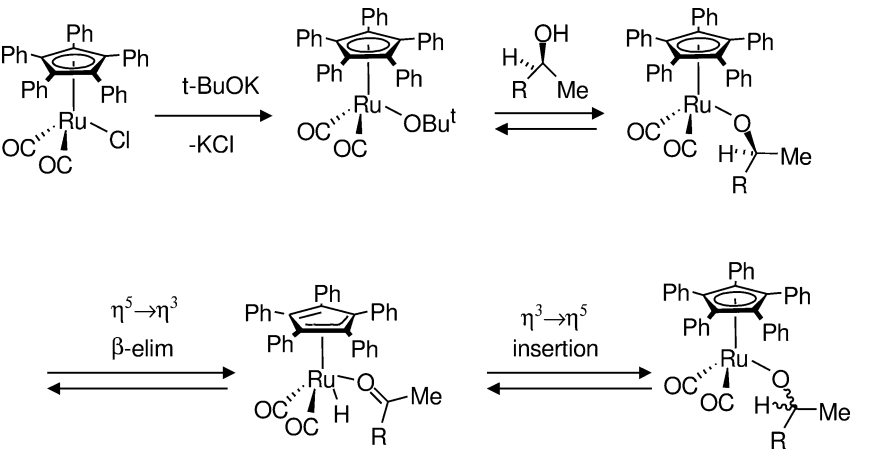


Fig. 9.7 Mechanism of Ru-catalyzed racemization of alcohols.

Notwithstanding these excellent results, there is still some room for further improvement. All of these systems require anaerobic conditions, owing to their air sensitivity, and consequently cannot be reused. Indeed all of these systems involve significant (from a cost point of view) amounts (2–5 mol%) of a homogeneous ruthenium catalyst, which is difficult to recycle. An interesting recent development in this context is the report, by Kim and coworkers [19], of an air-stable, recyclable catalyst that is applicable to DKR at room temperature (Fig. 9.8).

One place to look for good alcohol racemization catalysts is in the pool of catalysts that are used for hydrogen transfer reduction of ketones. One class of complexes that are excellent catalysts for the asymmetric transfer hydrogenation comprises the ruthenium complexes of monosulfonamides of chiral diamines developed by Noyori and coworkers [20, 21]. These catalysts have been used for the asymmetric transfer hydrogenation of ketones [20] and imines [21] (Fig. 9.9).

Hence, we reasoned that they should be good catalysts for alcohol (and amine) racemization but without the requirement for a chiral ligand. This indeed proved to be the case: ruthenium complex **6** containing the achiral diamine monosulfonamide ligand was able to catalyze the racemization of 1-phenylethanol [13]. Noyori and coworkers [22] had proposed that hydrogen transfer catalyzed by theses complexes involves so-called metal-ligand bifunctional catalysis, whereby hydrogens are transferred from both the ruthenium and the ligand (see Fig. 9.10).

By analogy, it seemed plausible that alcohol racemizations catalyzed by the same type of ruthenium complexes involve essentially the same mechanism (Fig. 9.11), in which the active catalyst is first generated by elimination of HCl by the added base. This 16e complex subsequently abstracts two hydrogens from the alcohol substrate to afford an 18e complex and a molecule of ketone. Reversal of these steps leads to the formation of racemic substrate.

This system was also shown to catalyze the DKR of 1-phenylethanol [13], interestingly in the presence of the stable free radical TEMPO as cocatalyst (Fig. 9.12). The exact role of the TEMPO in this system has yet to be elucidated and the reaction conditions need to be optimized, e.g. by preforming the active catalyst as in the Bäckvall system described above.

Similarly, the DKR of chiral amines can, in principle, be achieved by combining the known amine resolution by lipase-catalyzed acylation [23] with metal-cat-

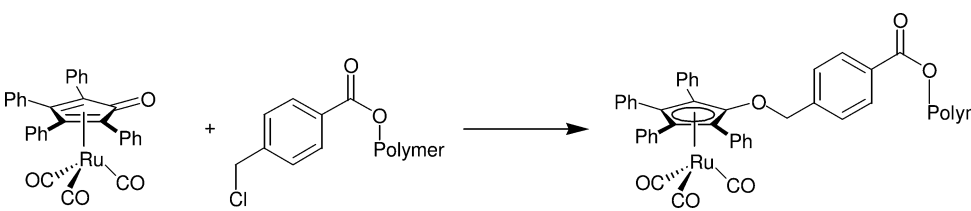


Fig. 9.8 Air stable, recyclable Ru catalyst for DKR of alcohols.

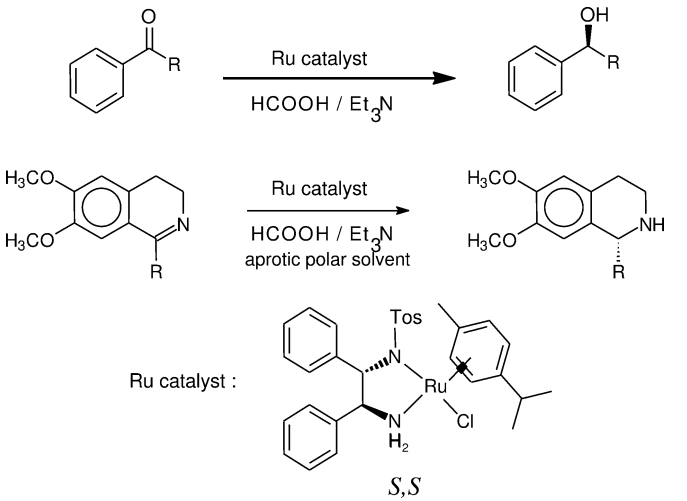


Fig. 9.9 Asymmetric transfer hydrogenation of ketones and imines.

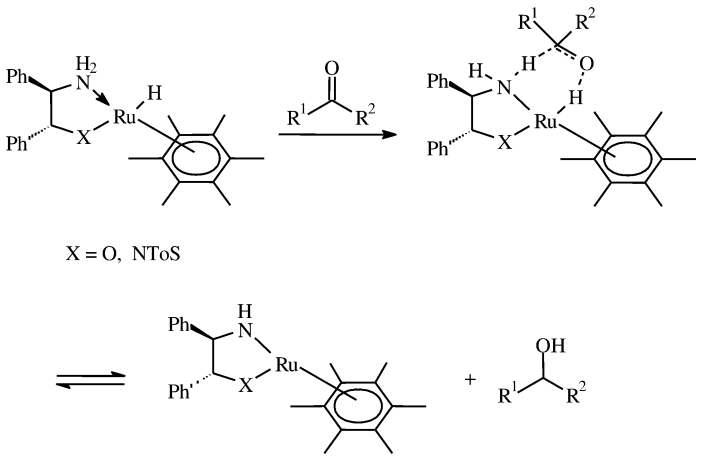


Fig. 9.10 Metal-ligand bifunctional catalysis.

alyzed racemization via a dehydrogenation/hydrogenation mechanism. However, although the DKR of alcohols is well established there are few examples of the DKR of amines. Reetz and Schimossek in 1996 reported the DKR of 1-phenethylamine (Fig. 9.13), using Pd-on-charcoal as the racemization catalyst [24]. However, the reaction was slow (8 days) and competing side reactions were responsible for a relatively low yield.

The racemization of amines is more difficult than that of alcohols owing to the more reactive nature of the intermediate (imine vs. ketone). Thus, imines readily undergo reaction with a second molecule of amine and/or hydrogenoly-

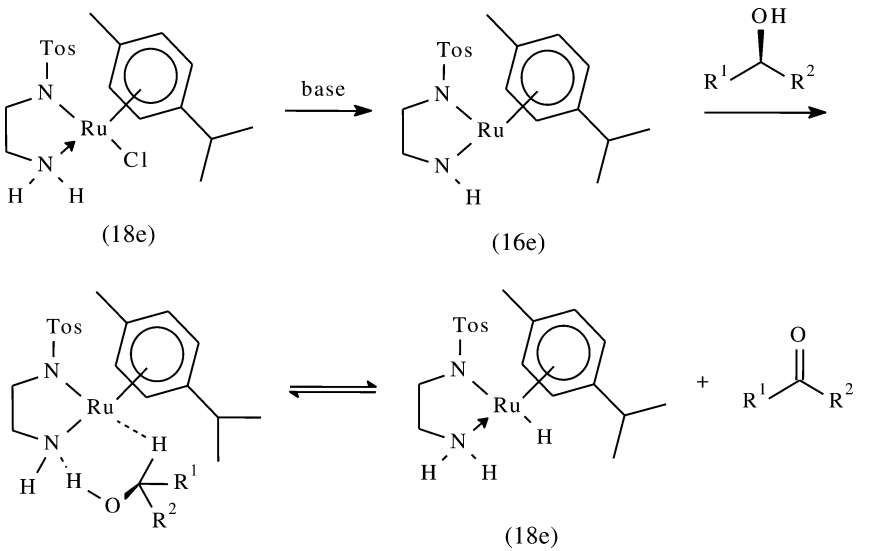


Fig. 9.11 Mechanism of racemization catalyzed by complex **6**.

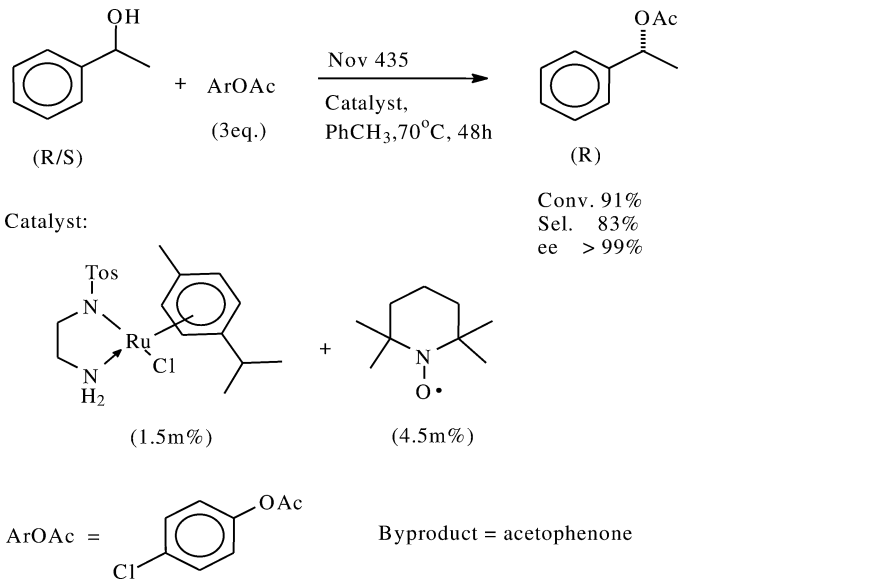


Fig. 9.12 DKR of 1-phenylethanol catalyzed by **6**/CaLB.

sis reactions as shown in Fig. 9.14. Furthermore, in the presence of water the imine can undergo hydrolysis to the corresponding ketone.

Recently, Jacobs and coworkers [25] showed that the use of alkaline earth supports, rather than charcoal, for the palladium catalyst led to substantial reduc-

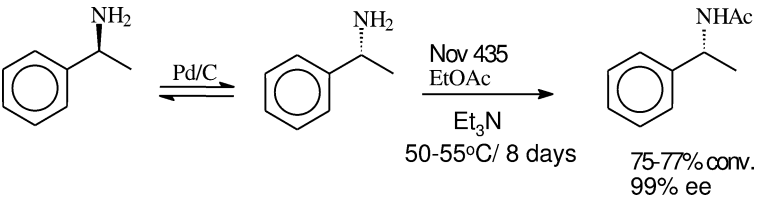


Fig. 9.13 DKR of 1-phenylethylamine catalyzed by a lipase/Pd-on-C.

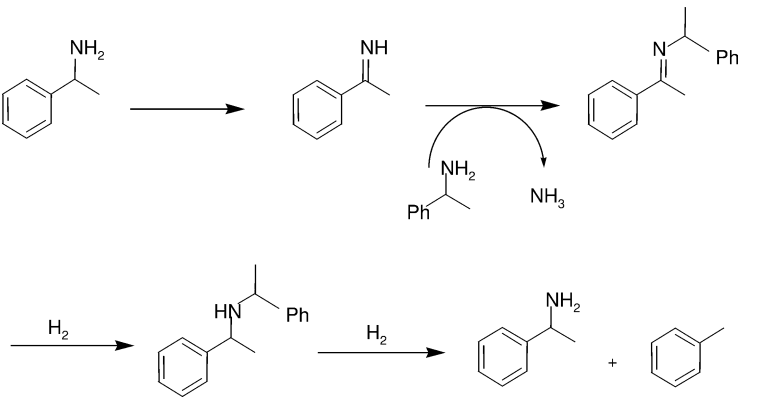
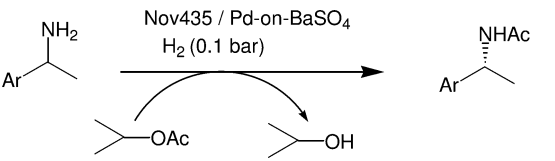


Fig. 9.14 Side reactions in metal-catalyzed racemization of amines.

tion in byproduct formation. It was suggested that the basic support suppresses the condensation of the amine with the imine (see Fig. 9.14). A 5% Pd-on-$BaSO_4$ catalyst was chosen for further study and shown to catalyze the DKR of amines with reaction times of 1–3 days at 70 °C. Good to excellent chemo- and enantioselectivities were obtained with a variety of chiral benzylic amines (see Fig. 9.15).



Ar	Time(h)	Conv.(%)	sel. amide (%)	ee (%)
C_6H_5	24	91	94	>99
4-MeC_6H_4	24	73	97	>99
4-MeOC_6H_4	48	90	98	>99
2-naphthyl	48	89	87	99
1-naphthyl	48	64	87	99

Fig. 9.15 DKR of primary amines catalyzed by Pd-on-$BaSO_4$/lipase.

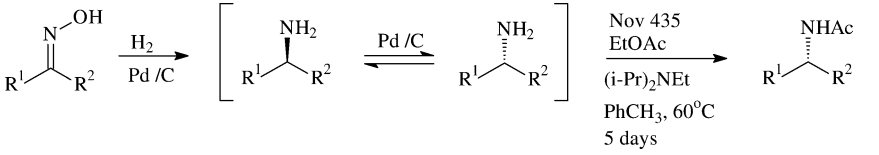


Fig. 9.16 Optically active amines from prochiral ketoximes.

Alternatively, Kim and coworkers showed [26] that prochiral ketoximes could be converted into optically active amines by hydrogenation over Pd-on-C in the presence of CaLB and ethyl acetate as both acyl donor and solvent, presumably via DKR of the amine formed *in situ* (see Fig. 9.16).

Paetzold and Backvall [27] have reported the DKR of a variety of primary amines using an analog of the ruthenium complex **1** as the racemization catalyst and isopropyl acetate as the acyl donor, in the presence of sodium carbonate at 90 °C (Fig. 9.17). Apparently, the function of the latter was to neutralize traces of acid, e.g. originating from the acyl donor, which would deactivate the ruthenium catalyst.

Another important difference between (dynamic) kinetic resolution of alcohols and amines is the ease with which the acylated product, an ester and an amide, respectively, is hydrolyzed. This is necessary in order to recover the substrate enantiomer which has undergone acylation. Ester hydrolysis is generally a facile process but amide hydrolysis, in contrast, is often not trivial. For example, in the BASF process [28] for amine resolution by lipase-catalyzed acylation the amide product is hydrolyzed using NaOH in aq. ethylene glycol at 150 °C (Fig. 9.18). In the case of phenethylamine this does not present a problem but it will obviously lead to problems with a variety of amines containing other functional groups.

We reasoned that, since an enzyme was used to acylate the amine under mild conditions, it should also be possible to employ an enzyme for the deacylation of the product. This led us to the concept of the Easy-on-Easy-off resolution of

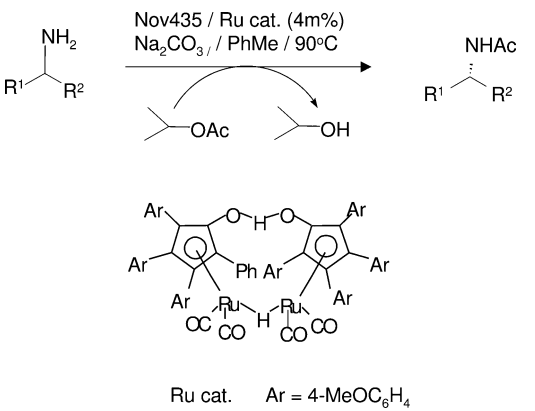


Fig. 9.17 DKR of amines catalyzed by Ru/CaLB.

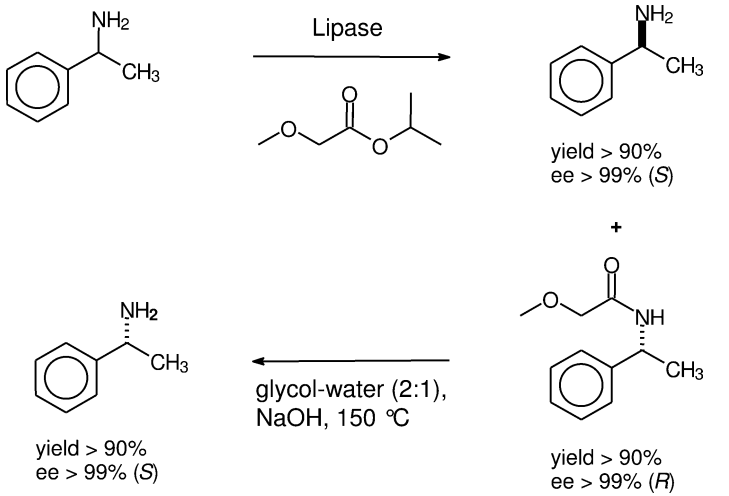


Fig. 9.18 Lipase-catalyzed resolution of amines: BASF process.

amines [29] which involves three steps: enzymatic acylation and deacylation and racemization of the unwanted enantiomer (Fig. 9.19). Combination of acylation and deacylation, in a DKR process as described above, leads to a two-step process in which only one enantiomer of the amine is formed.

For example, we showed that penicillin acylase from *Alcaligenes faecalis* could be used for acylation of amines in an aqueous medium at high pH. After extraction of the remaining amine isomer, the pH was reduced to 7 and the amide hydrolyzed to afford the other amine enantiomer (Fig. 9.20).

This one-pot process, performed in water, is clearly an attractive, green alternative to the above-mentioned BASF process. Unfortunately, high enantioselectivities are obtained only with amine substrates containing an aromatic moiety.

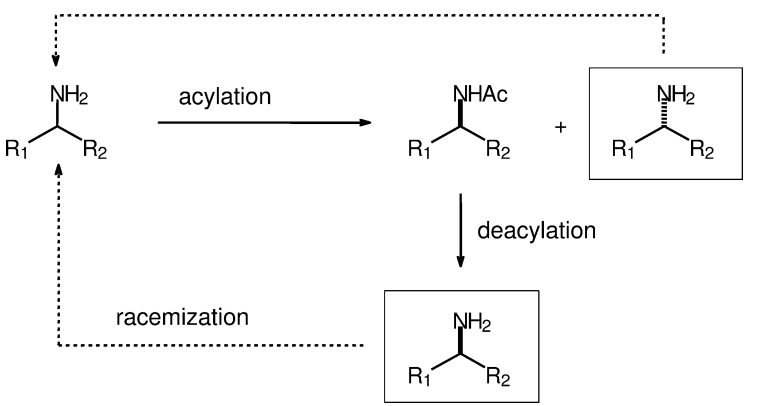


Fig. 9.19 The Easy-on-Easy-off resolution of amines.

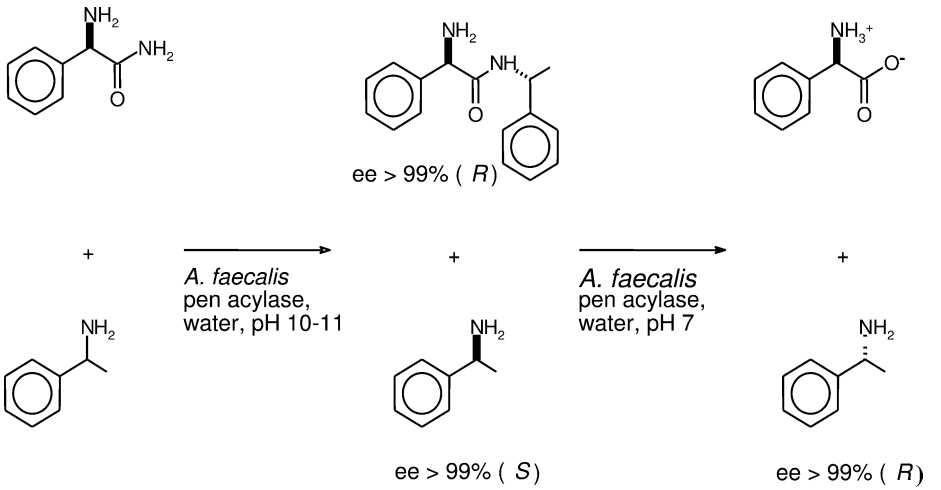


Fig. 9.20 Resolution of an amine with penicillin acylase.

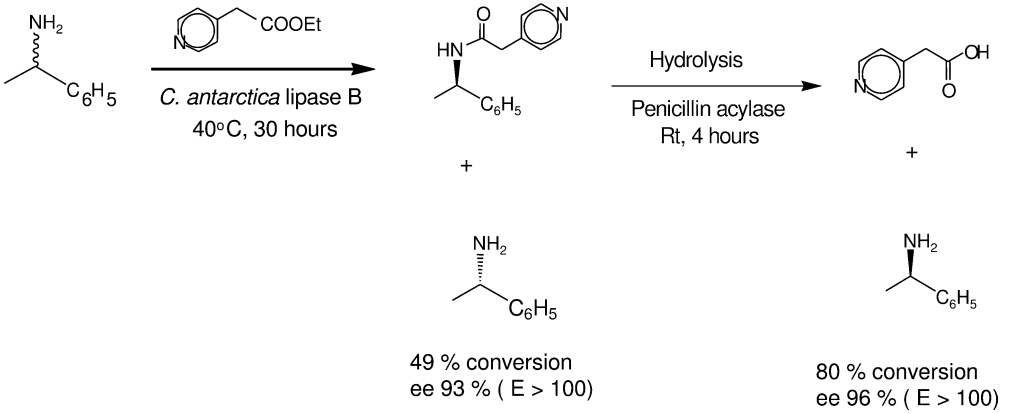


Fig. 9.21 Two-enzyme resolution of amines.

In order to broaden the scope we also examined [30] a combination of lipase-catalyzed acylation with penicillin acylase-catalyzed hydrolysis (deacylation). Good results (high enantioselectivity in the acylation and smooth deacylation) were obtained, with a broad range of both aliphatic amines and amines containing an aromatic moiety, using pyridylacetic acid ester as the acyl donor (Fig. 9.21).

We subsequently found that CaLB could be used for both steps (Fig. 9.22). That a lipase was able to effectively catalyze an amide hydrolysis was an unexpected and pleasantly surprising result. Good results were obtained with CaLB immobilized as a CLEA (see later).

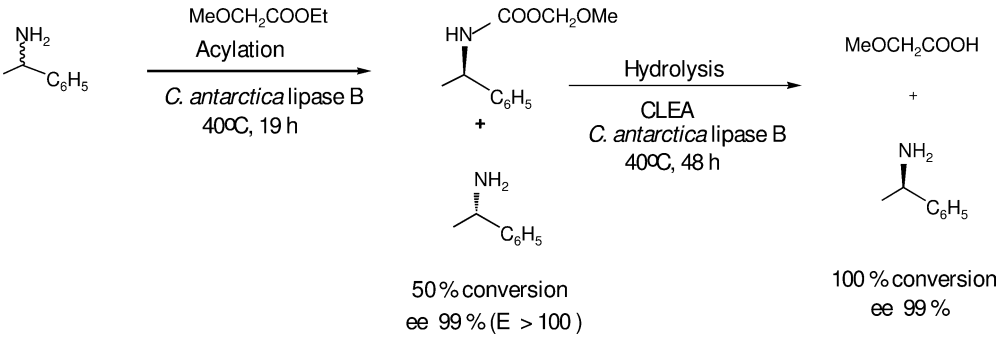


Fig. 9.22 CaLB for acylation and deacylation steps in amine resolution.

9.3
Combination of Asymmetric Hydrogenation with Enzymatic Hydrolysis

Asymmetric catalysis by chiral metal complexes is also an important technology for the industrial synthesis of optically active compounds [6, 31]. Most commercial applications involve asymmetric hydrogenation [31]. The classic example is the Monsanto process for the production of L-Dopa, developed in the 1960s [32]. Knowles was awarded the Nobel Prize in Chemistry for the development of this benchmark process in catalytic asymmetric synthesis. A closer inspection of the process (Fig. 9.23) reveals that, subsequent to the key catalytic asymmetric hydrogenation step, three hydrolysis steps (ester, ether, and amide hydrolysis), are required to generate the L-Dopa product. One can conclude, therefore, that there is still room for improvement e.g. by replacing classical chemical hydrolysis steps by cleaner, enzymatic hydrolyses under mild conditions, resulting in a reduction in the number of steps and the consumption of chemicals and energy. Furthermore, the homogeneous catalyst used in this and analogous

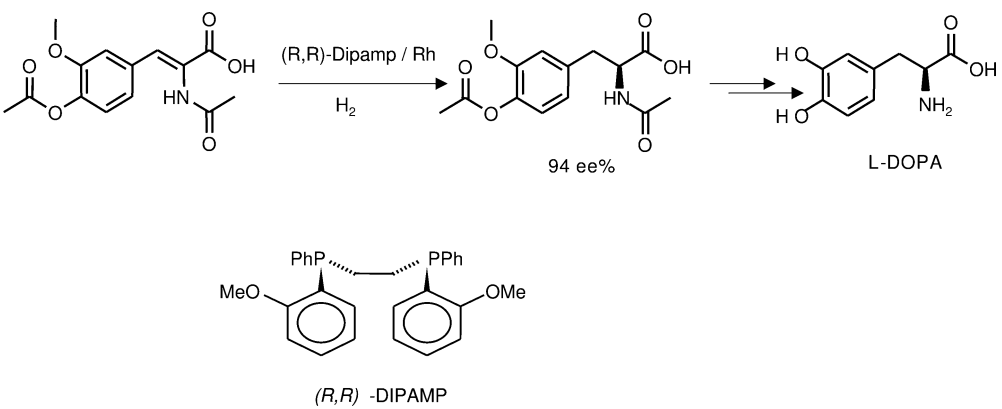


Fig. 9.23 Monsanto L-Dopa process.

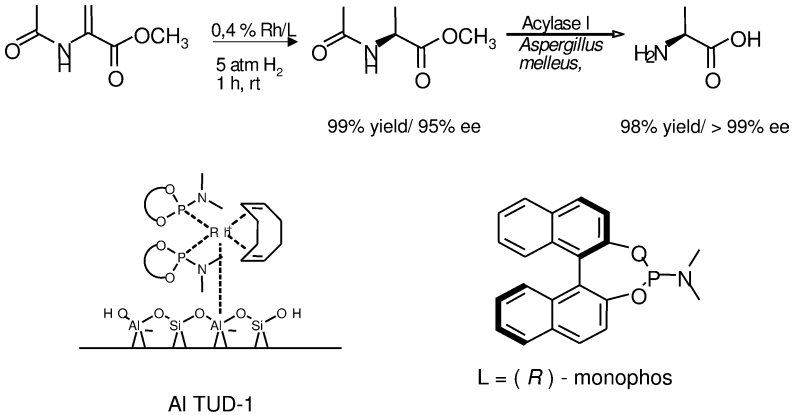


Fig. 9.24 Chemoenzymatic, one-pot synthesis of an amino acid.

asymmetric hydrogenations comprises two expensive components, a noble metal and a chiral diphosphine ligand. Hence, recovery and recycling of the catalyst is an important issue, as discussed in Chapter 7.

Consequently, much attention is currently being focused on telescoping such processes and integrating catalyst recovery and recycling into the overall process. For example, we have recently combined an analogous asymmetric hydrogenation of an *N*-acyl dehydroamino acid (Fig. 9.24), using a supported chiral Rh catalyst, with enzymatic hydrolysis of the product, affording a one-pot cascade process in water as the only solvent [33]. An additional benefit is that the enantiomeric purity of the product of the asymmetric hydrogenation is upgraded in the subsequent enzymatic amide hydrolysis step (which is highly selective for the desired enantiomer). The ester moiety in the intermediate also undergoes enzymatic hydrolysis under the reaction conditions.

9.4
Catalyst Recovery and Recycling

As noted above, recovery and recycling of chemo- and biocatalysts is important from both an economic and an environmental viewpoint. Moreover, compartmentalization (immobilization) of the different catalysts is a *conditio sine qua non* for the successful development of catalytic cascade processes. As discussed in Chapter 7, various approaches can be used to achieve the immobilization of a homogeneous catalyst, whereby the most well-known is heterogenization as a solid catalyst as in the above example.

An approach to immobilization which has recently become popular is microencapsulation in polymers, such as polystyrene and polyurea, developed by the groups of Kobayashi [34] and Ley [35], respectively. For example, microencapsulation of palladium salts or palladium nanoparticles in polyurea microcapsules

affords recyclable catalysts for carbonylations, Heck and Suzuki couplings and (transfer) hydrogenations [36].

Another approach to facilitating catalyst separation while maintaining the benefits of homogeneous catalysis involves the use of thermoregulated biphasic catalysis, as already mentioned in Chapter 7. In this approach the catalyst dissolves in a particular solvent at one temperature and is insoluble at another. For example, a ligand or an enzyme can be attached to an ethylene oxide/propylene oxide block copolymer (see Chapter 7) to afford catalysts that are soluble in water at room temperature but precipitate on warming to 40 °C. The driving force for this inverted temperature dependence on solubility is dehydration of the ligand on heating. An added advantage is that runaway conditions are never achieved since the catalyst precipitates and the reaction stops on raising the temperature. An example of the application of this technique to biotransformations involves the covalent attachment of penicillin acylase to poly-*N*-isopropylacrylamide (PNIPAM) [37].

An interesting example of the use of a recyclable, thermoresponsive catalyst in a micellar-type system was recently reported by Ikegami et al. [38]. A PNIPAM-based copolymer containing pendant tetraalkylammonium cations and a polyoxometalate, $PW_{12}O_{40}^{3-}$, as the counter anion was used as a catalyst for the oxidation of alcohols with hydrogen peroxide in water (Fig. 9.25). At room temperature the substrate and the aqueous hydrogen peroxide, containing the catalyst, formed distinct separate phases. When the mixture was heated to 90 °C a

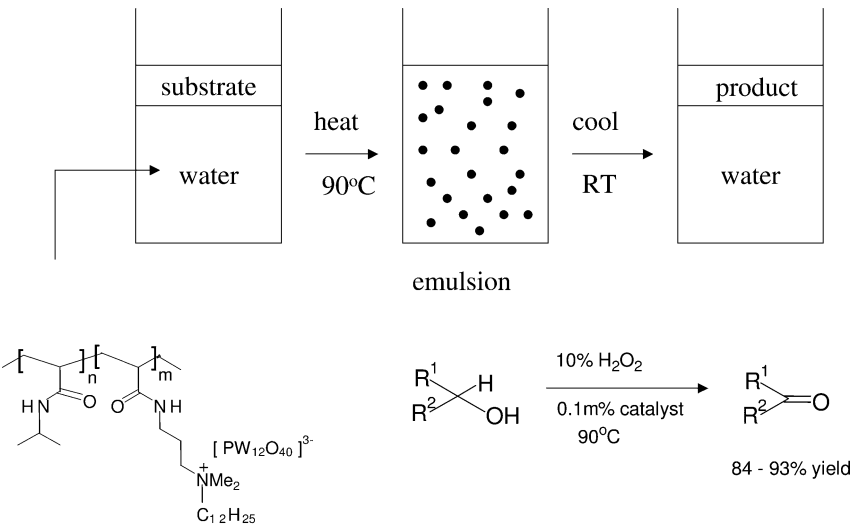


Fig. 9.25 Oxidation of alcohols with hydrogen peroxide using a thermoresponsive catalyst in a micellar system.

stable emulsion was formed, in which the reaction took place with as little as 0.1 mol% catalyst. Subsequent cooling of the reaction mixture to room temperature resulted in precipitation of the catalyst, which could be removed by filtration and recycled.

Another interesting concept involves so-called organic aqueous tunable solvents (OATS) for performing (bio)catalytic processes [39]. The reaction takes place in a single liquid phase, comprising a mixture of water and a water miscible organic solvent, such as tetrahydrofuran or dioxane. When the reaction is complete, carbon dioxide is added to generate a biphasic system consisting of a gas-expanded liquid organic phase containing hydrophobic components and an aqueous phase containing the hydrophilic (bio)catalyst.

This is reminiscent of the 'miscibility switch', described in Chapter 7, whereby carbon dioxide and an ionic liquid are miscible at one pressure and become immiscible on reducing the pressure. This provided the possibility of performing an asymmetric hydrogenation in a monophasic ionic liquid/carbon dioxide mixture and, when the reaction is complete, reducing the pressure to afford a biphasic mixture in which the product is in the carbon dioxide phase and the rhodium catalyst in the ionic liquid phase, which can be recycled [40]. Another variation on this theme is a smart solvent that switches reversibly from a liquid with one set of properties to another with different properties. In the example presented, a mixture of an alcohol and an amine was allowed to react with carbon dioxide to generate an ionic liquid (Fig. 9.26) which reverts back to its non-ionic form when purged with nitrogen or argon [41].

In Chapter 7 we have already discussed the use of fluorous biphasic systems to facilitate recovery of catalysts that have been derivatized with fluorous "ponytails". The relatively high costs of perfluoroalkane solvents coupled with their persistent properties pose serious limitations for their industrial application. Consequently, second generation methods have been directed towards the elimination of the need for perfluoro solvents by exploiting the temperature-dependent solubilities of fluorous catalysts in common organic solvents [42]. Thus, appropriately designed fluorous catalysts are soluble at elevated temperatures and essentially insoluble at lower temperatures, allowing for catalyst recovery by simple filtration.

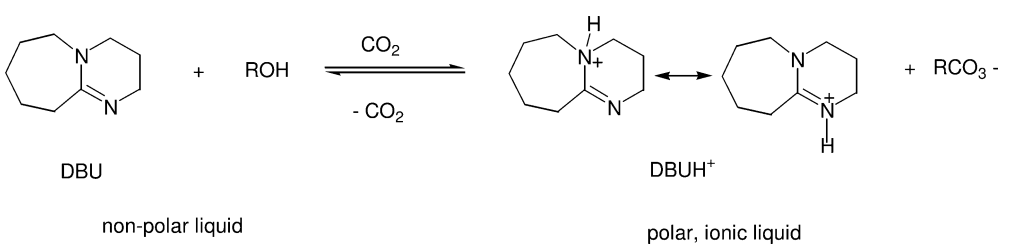


Fig. 9.26 Reversible non-polar to polar solvent switch.

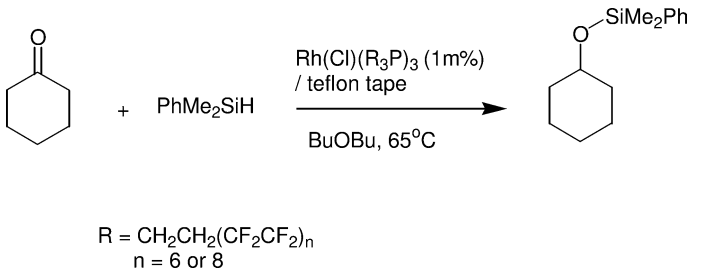


Fig. 9.27 Catalyst-on-Teflon-tape for recycling of fluorous catalysts.

Alternatively, an insoluble fluorous support, such as fluorous silica [43], can be used to adsorb the fluorous catalyst. Recently, an eminently simple and effective method has been reported in which common commercial Teflon tape is used for this purpose [44]. This procedure was demonstrated with a rhodium-catalyzed hydrosilylation of a ketone (Fig. 9.27). A strip of Teflon tape was introduced into the reaction vessel and when the temperature was raised the rhodium complex, containing fluorous ponytails, dissolved. When the reaction was complete the temperature was reduced and the catalyst precipitated onto the Teflon tape which could be removed and recycled to the next batch.

9.5 Immobilization of Enzymes: Cross-linked Enzyme Aggregates (CLEAs)

We have recently developed an extremely effective method for immobilizing enzymes as so-called Cross-Linked Enzyme Aggregates (CLEAs) [45]. They exhibit high activity retention and stability and can be readily recovered and recycled without any loss of activity. Furthermore, the method is exquisitely simple – precipitation from aqueous buffer followed by cross-linking with, for example, glutaraldehyde – and is applicable to a broad range of enzymes. It does not require highly pure enzyme preparations and it actually constitutes a combination of purification and immobilization into one step. The methodology can also be applied to the co-immobilization of two or more enzymes to give 'combi CLEAs' which are more effective than mixtures of the individual CLEAs. These are ideally suited to conducting enzymatic cascade reactions in water, where an equilibrium can be shifted by removing the product in a consecutive biotransformation. For example, we have used a combi CLEA containing an *S*-selective nitrilase (from *Manihot esculenta*) and a non-selective nitrilase, in DIPE/Water (9:1) at pH 5.5, 1 h, for the one-pot conversion of benzaldehyde to *S*-mandelic acid (Fig. 9.28) in high yield and enantioselectivity [46].

A CLEA prepared from CaLB was recently shown to be an effective catalyst for the resolution of 1-phenylethanol and 1-tetralol in supercritical carbon dioxide in continuous operation [47]. Results were superior to those obtained with Nov 435 (CaLB immobilized on a macroporous acrylic resin) under the same

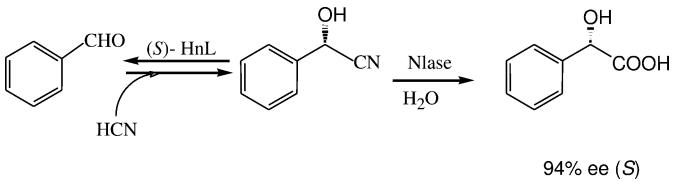


Fig. 9.28 One-pot conversion of benzaldehyde to *S*-mandelic acid with a combi CLEA.

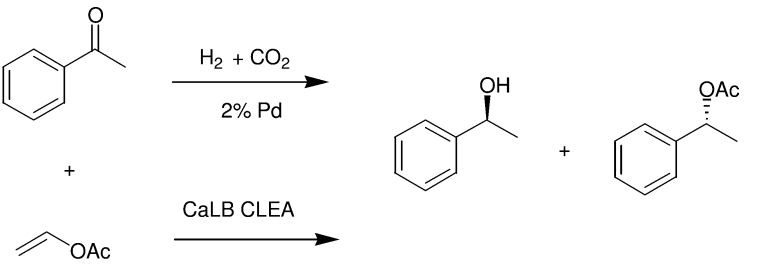


Fig. 9.29 Continuous kinetic resolution catalyzed by a CaLB CLEA in $scCO_2$.

conditions. In addition, the palladium-catalyzed hydrogenation of acetophenone and the resolution of the resulting 1-phenylethanol were performed in series (Fig. 9.29), thereby achieving a reduction in energy consumption by obviating the need for de- and re-pressurization between the steps.

9.6 Conclusions and Prospects

The key to sustainability is catalysis and success will be dependent on an effective integration of catalysis in organic synthesis, including downstream processing. Traditional barriers between catalysis and mainstream organic synthesis are gradually disappearing, as are the traditional barriers separating the sub-disciplines of catalysis: heterogeneous, homogeneous and enzymatic. Hence, there is a trend towards the development of multistep syntheses involving a variety of catalytic steps. The ultimate in efficiency is to telescope these syntheses into catalytic cascade processes. A common problem encountered in the design of such processes is that of incompatibility of the different catalysts. A possible solution is to follow the example of Nature: compartmentalization as the key to compatibility. In practice, this means immobilization of the catalyst, which at the same time provides for its efficient recovery and recycling. As we have seen, various approaches can be envisaged for achieving this immobilization, including not

only traditional immobilization as a filterable solid catalyst but also 'smart methods' such as attachment of catalysts to thermoresponsive polymers.

Finally, it is worth mentioning that a successful integration of catalytic reaction steps with product separation and catalyst recovery operations will also be dependent on innovative chemical reaction engineering. This will require the widespread application of sustainable engineering principles [48].In this context 'process intensification', which involves the design of novel reactors of increased volumetric productivity and selectivity with the aim of integrating different unit operations to reactor design, and miniaturization will play pivotal roles [49, 50].

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10
Epilogue: Future Outlook

10.1
Green Chemistry: The Road to Sustainability

There is no doubt that green chemistry is here to stay. Chemical companies and, indeed, companies in general are placing increasing emphasis on sustainability and environmental goals in their corporate mission statements and annual reports. The European Technology Platform on Sustainable Chemistry (SusChem) has recently published an Implementation Action Plan entitled "Putting Sustainable Chemistry into Action" [1]. The report defines three key technology areas: (i) industrial biotechnology, (ii) materials technology, and (iii) reaction and process design. The goal is "improving the eco-efficiency of products and processes to optimize the use of resources and minimize waste and environmental impact". Similarly, the US chemical industry produced a strategic plan – Technology Vision 2020 – which defined a long-term technology roadmap for the future [2]. Important goals were to "improve efficiency in the use of raw materials, the reuse of recycled materials, and the generation and use of energy and to continue to play a leadership role in balancing environmental and economic considerations".

To readers of this book this will all sound rather familiar. It contains an underlying and unifying theme of green chemistry as the means for achieving these noble goals. Initially, many people confused green chemistry with what is generally known as environmental chemistry which is concerned with the effects of chemicals on the environment and remediation of waste and contaminated land and water. In contrast, green chemistry is concerned with redesigning chemical products and processes to avoid the generation and use of hazardous substances and the formation of waste, thus obviating the need for a lot of the environmental chemistry.

The twelve principles of green chemistry, as expounded by Anastas and Warner in 1998 [3], have played an important role in promoting its application. They inspired others to propose additional principles [4] and, more recently, Anastas and Zimmerman [5] proposed the twelve principles of green engineering which embody the same underlying features – conserve energy and resources and avoid waste and hazardous materials – as those of green chemistry, but from an engineering viewpoint. More recently, a mnemonic, PRODUC-

P – Prevent waste
R – Renewable materials
O – Omit derivatization steps
D – Degradable chemical products
U – Use safe synthetic methods
C – Catalytic reagents
T – Temperature, pressure ambient
I – In- process monitoring
V – Very few auxiliary substances
E – E-factor, maximize feed in product
L – Low toxicity of chemical products
Y – Yes, it is safe

Fig. 10.1 Condensed principles of green chemistry.

TIVELY, has been proposed which captures the spirit of the twelve principles of green chemistry and can be presented as a single slide (Fig. 10.1) [6].

In the USA the Presidential Green Chemistry Challenge Awards [7] were introduced to stimulate the application of the principles of green chemistry and many chemical and pharmaceutical companies have received awards for the development of greener processes and products, e.g. Pfizer for developing a greener process for sildenafil manufacture (see Chapter 7).

Graedel [8] has reduced the concept of green chemistry and sustainable development to four key areas: (i) sustainable use of chemical feedstocks, (ii) sustainable use of water, (iii) sustainable use of energy and (iv) environmental resilience. These reflect the central tenets of sustainability, that is, "using natural resources at rates that do not unacceptably draw down supplies over the long term and (ii) generating and dissipating residues at rates no higher than can be assimilated readily by the natural environment".

10.2
Catalysis and Green Chemistry

Hopefully, this book has made clear that there is a common technological theme which underlies these concepts of green chemistry and sustainability in chemical products and processes, and that is the application of catalysis – homogeneous, heterogeneous and enzymatic. The application of catalytic technologies leads to processes that are more efficient in their use of energy and raw materials and generate less waste. The importance of catalysis in this context was recently underlined by the award of the 2005 Nobel Prize in Chemistry to Grubbs, Schrock and Chauvin for the development of the olefin metathesis reaction, a classic example of clean catalytic chemistry. According to the Swedish Academy olefin metathesis is "a great step forward for green chemistry". Similarly, the 2001 Nobel Prize in Chemistry was awarded to Noyori and

Knowles and Sharpless, for catalytic asymmetric hydrogenation and asymmetric oxidation, respectively.

In the future we can expect a shift towards the use of renewable raw materials which will completely change the technological basis of the chemical industry. It will require the development of catalytic processes, both chemo- and biocatalytic, for the conversion of these raw materials to fuels and chemicals. New coproducts will be generated which in turn can serve as feedstocks for other chemicals. A pertinent example is biodiesel manufacture which generates large amounts of glycerol, thus creating a need for new (catalytic) processes for the conversion of glycerol to useful products (see Chapter 8).

The shift towards biomass as a renewable raw material will also result in the development of alternative, greener products, e.g. biodegradable polymers, polymers derived from biomass to replace poorly degradable synthetic polymers. A good example of this is the production of carboxystarch by oxidation of starch. The product is a biodegradable water super absorbent which could replace the synthetic polyacrylate-based super adsorbents which have poor biodegradability. The traditional process to produce carboxystarch involves TEMPO catalyzed oxidation of starch with sodium hypochlorite, which generates copious amounts of sodium chloride as a waste product and possibly chlorinated byproducts. There is a need, therefore, for a greener, catalytic process using hydrogen peroxide or dioxygen as the oxidant.

It has been shown [9] that the copper-dependent oxidase enzyme, laccase, in combination with TEMPO or derivatives thereof, is able to catalyze the aerobic oxidation of the primary alcohol moieties in starch (Fig. 10.2). There is currently considerable commercial interest in laccases for application in pulp bleaching (as a replacement for chlorine) in paper manufacture and remediation of phenol-containing waste streams [10].

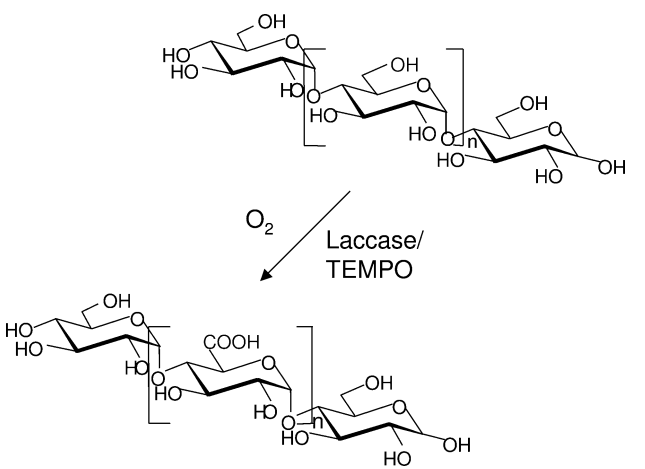

Fig. 10.2 Oxidation of starch to carboxystarch.

However, the relatively high enzyme costs form an obstacle to commercialization. Inefficient laccase use is a result of its instability towards the oxidizing reaction conditions. We have recently shown that the stability of the laccase under reaction conditions can be improved by immobilization as a cross-linked enzyme aggregate (see Chapter 9). It has also been shown that a water-soluble iron complex of a sulfonated phthalocyanine ligand is an extremely effective catalyst for starch oxidation with hydrogen peroxide in an aqueous medium [11].

It is also worth mentioning in this context that in the last few years a separate sub-division of catalysis has been gathering momentum: organocatalysis [12]. A wide variety of (enantioselective) reactions can be catalyzed and homogeneous organocatalysts can be heterogenized, on solid supports or in liquid/liquid biphasic systems, using similar methodologies to those used for metal complexes (see earlier chapters).

10.3 The Medium is the Message

As discussed at length in Chapter 7, the reaction medium is an important issue in green chemistry from two points of view: (i) the problem of solvent emissions and (ii) the problem of product separation and catalyst recovery and recycling. Many innovative approaches are being followed to solve these problems (see Chapters 7 and 9). Water and carbon dioxide have, in the grand scheme of things, obvious advantages and, in this context, it is worth mentioning the recent report on chemistry "on water" [13]. Several uni- and bimolecular reactions were greatly accelerated when performed in vigorously stirred aqueous suspensions. The efficiency is quite amazing but the origin of the rate enhancements remains unclear. Both heterogeneity and water appear to be required and the hydration layers of the particles may play a crucial role. We note, however, that the technique of so-called solid-to-solid transformations is quite often used for biocatalytic processes in water where both substrate and product are sparingly soluble in water [14]. Organocatalysis (see above) also lends itself, in some instances, to operation in aqueous media and recent reports include the use of praline-based organocatalysts for enantioselective aldol reactions [15] and Michael additions [16] in water. Even more simple is the recent report of asymmetric aldol reactions catalyzed by 10 mol% of the amino acid, tryptophan, in water as the sole solvent [17]. High enantioselectivities were obtained in the aldol reaction of a variety of cycloalkanones with aromatic aldehydes (see Fig. 10.3 for an example).

The use of ionic liquids as reaction media is undergoing an exponential growth with the emphasis shifting to the use of non-toxic, biodegradable ionic liquids, preferably from renewable raw materials [18]. There is even a IUPAC ionic liquids data base, "IL Thermo" [19].

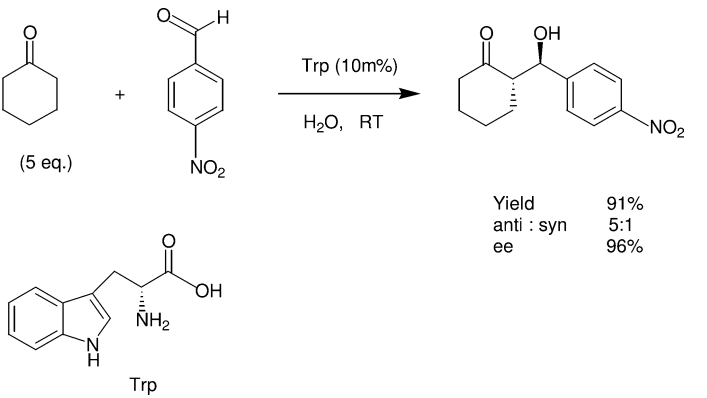

Fig. 10.3 Asymmetric aldol reaction catalyzed by tryptophan in water.

10.4
Metabolic Engineering and Cascade Catalysis

The need for novel catalytic processes is clear and, as discussed in Chapter 9, combining catalytic steps into cascade processes, thus obviating the need for isolation of intermediate products, results in a further optimization of both the economics and the environmental footprint of the process. *In vivo* this amounts to metabolic pathway engineering [20] of the host microorganism (see Chapter 8) and *in vitro* it constitutes a combination of chemo- and/or biocatalytic steps in series and is referred to as cascade catalysis (see Chapter 9). Metabolic engineering involves, by necessity, renewable raw materials and is a vital component of the future development of renewable feedstocks for fuels and chemicals.

10.5
Concluding Remarks

With sustainability as the driving force, the production and applications of chemicals are undergoing a paradigm change in the 21st century and green chemistry and catalysis are playing a pivotal role in this change. This revolutionary development manifests itself in the changing feedstocks for fuels and chemicals, from fossil resources to renewable feedstocks, and in the use of green catalytic processes for their conversion. In addition, there is a marked trend towards alternative, greener products that are less toxic and readily biodegradable. Ultimately this revolution will enable the production of materials of benefit for society while, at the same time, preserving the earth's precious resources and the quality of our environment for future generations.

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