ASYMMETRIC SYNTHESES AND TRANSFORMATIONS – TOOLS FOR CHIRALITY MULTIPLICATION IN DRUG SYNTHESIS

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Abstract: A review of currently used methods for the synthesis and resolution of enantiomers of drugs and their precursors is presented. For the synthesis part the methods of diastereoselective as well as enantioselective synthesis are discussed, with particular consideration given to enantioselective catalysis with either metal complexes or biocatalysts. Desymmetrization processes are also included as methods to access enantiomerically pure compounds. Racemate resolution still remains an important method to obtain pure enantiomers and methods involving kinetic resolution in enzymatic or chemical systems, and particularly in connection with racemization (dynamic kinetic resolution) are on the rise in fine chemical industry, when applicable.

Keywords: asymmetric synthesis, chiral auxiliary, chiral pool, chiral reagent, desymmetrization, drugs, enantiomers, diastereoselective synthesis, kinetic resolution, resolution.

The role of chirality in drug development is on the rise in the past forty years, ever since the thalidomide case has triggered interest in the interactions of individual stereoisomers with bioreceptors. The demand for ready access to stereoisomers (both diastereoisomers and enantiomers) of drug molecules has stimulated in turn basic research in the field of stereoselective synthesis (either diastereoselective or enantioselective). As a result of developments in the synthesis of pure enantiomers, as well as the recognition of differences in diastereoselective interactions of enantiomers with chiral receptors and due to strict procedures for drug registration, a large and increasing proportion of new drugs are now made of enantiopure ingredients. Indeed, currently more than 50% of commercial drugs have at least one stereogenic center and among the top ten best selling drugs nine are composed of chiral molecules, among them five are single enantiomers and only one is achiral (Table 1) (1).

The ways to secure enantiomerically pure substances is either through *chirality multiplication* or *chirality sorting*. The first term embraces both enantioselective (**a**, **b**) and diastereoselective (**e**) syntheses, whereas the other deals with racemates resolution by either chemical (**c**) or physical (**d**) methods (Scheme 1).

Enantioselective processes (**a**, **b**), such as asymmetric dihydroxylation (AD), asymmetric aldol reaction (AA), asymmetric epoxidation (AE), asymmetric hydrogenation (AH), asymmetric ring opening reactions (ARO) and asymmetric Diels-Alder reactions (ADA) are among the ultimate achievements of fine organic synthesis. Indeed, the 2001 Nobel Prize in chemistry was a tribute to spectacular developments in enantioselective synthesis due to the work of three eminent chemists, W.S. Knowles, R. Noyori and K. B. Sharpless. Their work has stimulated further efforts in enantioselective synthesis and its applications in pharmaceutical and agrochemical industries. A comparison of various methods for securing single enantiomers is given in Table 2.

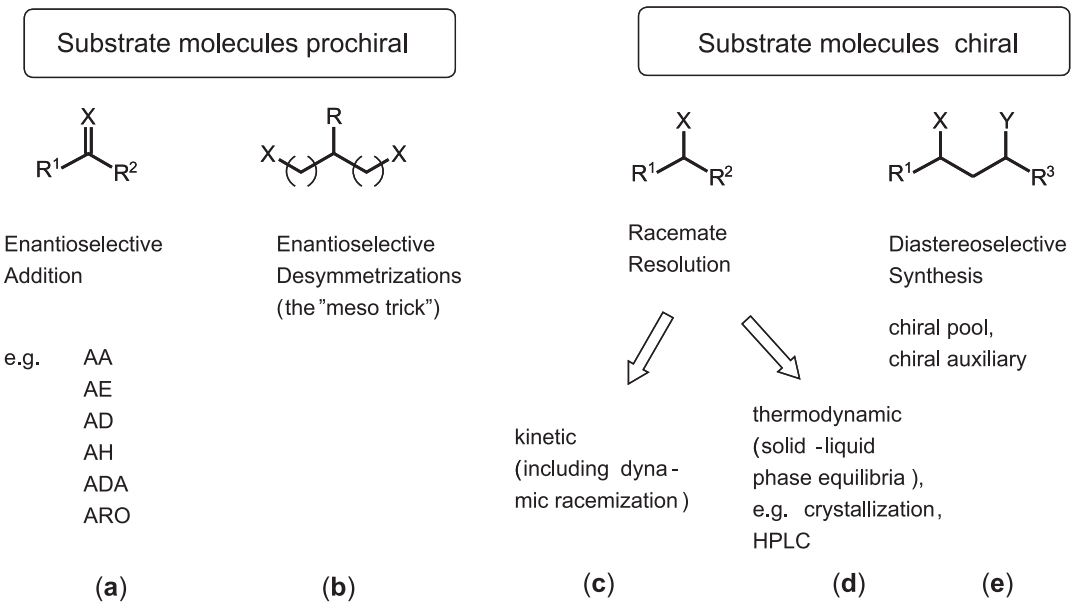
Diastereoselective syntheses involve syntheses of enantiomerically pure compounds from the pool of enantiomers available from the Nature (or *chiral pool)*, e.g. from amino acids, hydroxy acids, sugars, terpenes,

Table 1. Top ten best-selling drugs.

DRUG	GLOBAL 2004 SALES ($ BILLIONS)	ACTIVE INGREDIENT(S)	FORM OF ACTIVE INGREDIENT(S)	THERAPY CLASS
Lipitor	12.0	Atorvastatin	Single enantiomer	Cholesterol reducer
Zocor	5.9	Simvastatin	Single enantiomer	Cholesterol reducer
Plavix	5.0	Clopidogrel	Single enantiomer	Antithrombotic
Nexium	4.8	Esomeprazole	Single enantiomer	Antiulcerant
Zyprexa	4.8	Olanzapine	Achiral	Antipsychotic
Norvasc	4.8	Amlodipine	Racemate	Antihypertensive
Seretide/Advair	4.7	Salmeterol Fluticasone	Racemate Single enantiomer	Bronchodilator Anti-inflammatory
Erypo	4.0	Epoetin alpha	Protein	Red blood cell stimulant
Ogastro	3.8	Lansoprazole	Racemate	Antiulcerant
Effexor	3.7	Venlafaxine	Racemate	Antidepressant

Table 2. Survey of methods for securing single enantiomers.

	Chiral pool	Chiral auxiliary	Chiral reagent	Chiral catalysis (biocatalysis)	Dynamic kinetic resolution	Enantiomers separation		
						by crystalization	by HPLC, SMBC, SFC	by kinetic resolution
Control of enantioselectivity	+	?	+	+	+	+	+	+
Atom economy	?	?	–	?	+	–	–	–
Generality	?	–	–	+	–	+	+	+
Scale-up	+	?		+	+	+		+



Scheme 1. Chirality multiplication (**a**, **b**, **e**) and sorting (**c**, **d**).

or alkaloids. Other variants of diastereoselective syntheses include the use of *chiral auxiliary* molecules (these can be either from chiral pool or synthetic). Enantioselective syntheses, now of increasing importance, are based on chiral catalysis. Chiral catalysts embrace a wide range of molecules, either metal complexes with chiral ligands, chiral organocatalysts (no metal involved) or chiral biocatalysts (enzymes). Less employed is a variant of enantioselective synthesis based on the use of *chiral reagents*. Separation of enantiomers from racemates is still an important method, particularly on industrial scale. Apart from rather traditional methods, such as crystallization, new techniques are gaining importance and applicability. These include high performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC) and simulated moving bed chromatography (SMBC) on chiral phases. *Kinetic resolution* of racemates, most often with the use of enzymes, takes the advantage of differences in reaction rates between two enantiomers. This is a particularly efficient method when coupled with the process of racemization (*dynamic kinetic resolution*) since it allows in principle to convert the whole racemate into just one enantiomer.

No single method of producing enantiomerically pure compounds is ideal. Table 2 shows some advantages (+) or disadvantages (-) of each method listed, addressing issues such as control of enantioselectivity, atom economy, generality and scale-up capability from medicinal chemistry to process chemistry. Whereas general assessment, although arbitrary, is possible in most cases, the performance of some methods (e. g. the use of chiral auxiliary) is strongly case sensitive. Further issues, such as environment impact, cost, recovery of chiral catalyst or chiral auxiliary, are of great importance at the production scale but again, these issues can best be assessed when considering each individual chiral target compound.

The various methods for chirality multiplication used in the synthesis of drug substances will be exemplified in the following sections.

Chiral pool

Naturally occurring, enantiomerically pure compounds are convenient substrates for chirality multiplication processes leading to drug substances. α-Amino acids are frequently used in production of chiral compounds, due to their ready availability, low cost and simple structure. L-Aspartic acid is an example of chiral pool member, used in the synthesis of imipenem (Merck), Scheme 2 (2).

Imipenem, a broad-spectrum carbapenem antibiotic, has three stereogenic centers of strictly defined configuration. L-Aspartic acid is a precursor of (now com-

mercially available) acetoxyazetidinone (**1**). In this intermediate the stereogenic center of aspartic acid is transformed to a new one having C-O, C-N bonds and two further centers of chirality are created. Lewis acid catalyzed substitution affords the diazointermediate **2** with retention of configuration. Rh-catalyzed diazoinsertion reaction provides the carbapenem **3** in which the three stereogenic centers of imipenem are preserved. The process outlined in Scheme 2 is remarkable for its atom economy and low environment impact.

Tadalafil (Cialis®) is synthesized by the process developed at ICOS/Eli Lilly, starting from D-tryptophan methyl ester (Scheme 3) (3, 4).

The process illustrates the situation where two diastereomeric products, **4**-*cis* and **4**-*trans*, of condensation of the amino acid ester with piperonal, are obtained and separated by crystallization. Tadalafil is obtained from **4**-*cis* isomer whereas **4**-*trans* diastereoisomer can be equilibrated under acidic conditions to the desired **4**-*cis* isomer.

Not only natural products form the chiral pool. Chiral synthetic compounds, such as glycidols, are often used as starting materials in drug synthesis. For example, antibacterial linezolid is obtained from (*R*)-glycidyl butyrate (**5**) by a process developed at Upjohn (Scheme 4) (5, 6).

Compound **5** is available industrially by lipase-catalyzed kinetic resolution of racemic glycidyl butyrate. Examples of other industrially important glycidols and epichlorohydrins from the chiral pool are shown in Scheme 5 (7).

Chiral auxiliary

Diastereoselective synthesis with the use of chiral auxiliary appears less favored in contemporary drug synthesis. The limiting factor is the recovery of chiral auxiliary, if it is a high cost substance. A remarkably highly diastereoselective aldol reaction of aldehyde **6** with chirally substituted acetate ester **7** to give aldol **8** is an example of viability of such an approach. Chiral alcohol part in the acetate ester **7** is a derivative of (*S*)-mandelic acid. After removal of the chiral auxiliary, followed by acetoacetate condensation and diastereoselective reduction of the keto group (+)-atorvastatin lactone was obtained, with two new centers of chirality and over 99% enantiomer excess (Scheme 6) (8, 9).

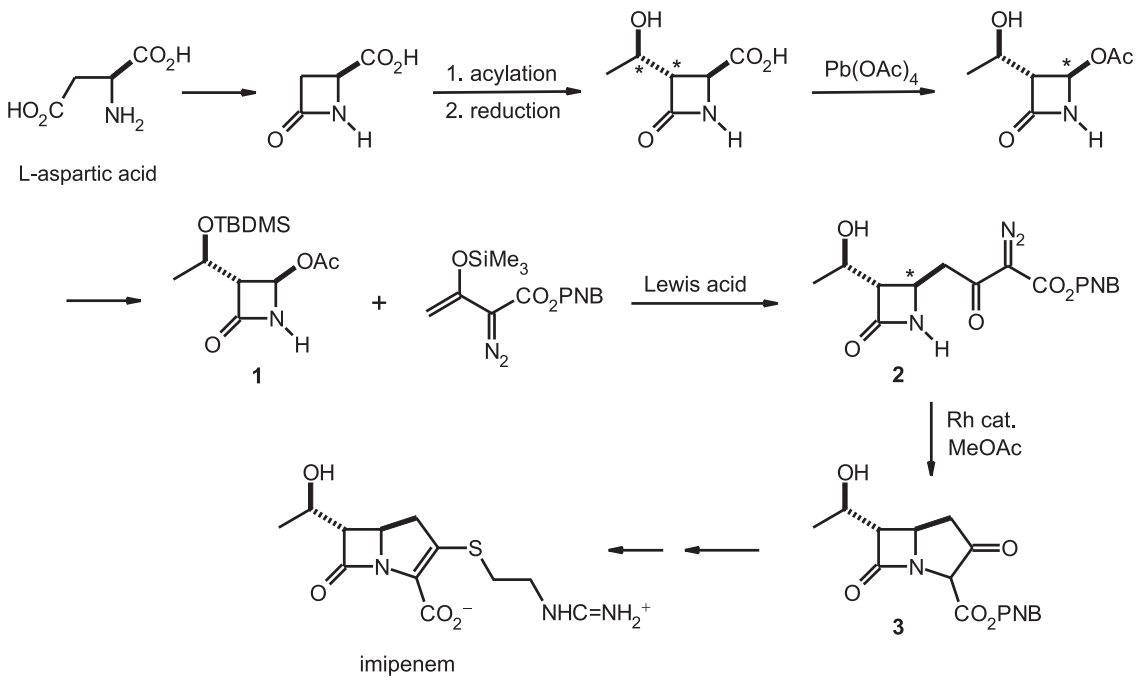
The calcium salt of atorvastatin (Lipitor®) is the enzyme HMG-CoA reductase inhibitor, used for treatment of hypercholesterolemia.

Chiral reagents

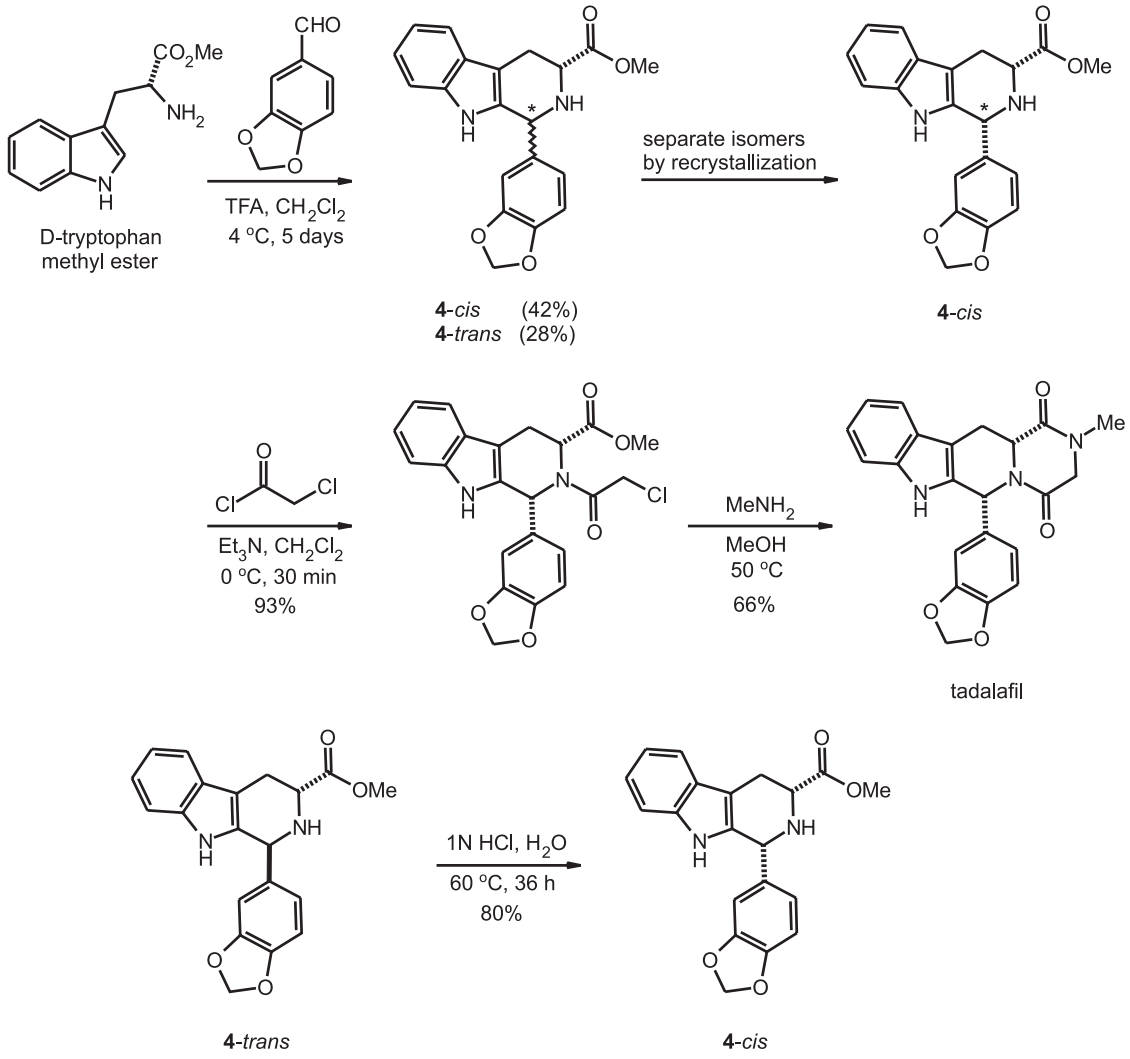
Asymmetric additions with the use of chiral reagents remain of interest for medicinal chemistry despite relatively high cost of chiral reagents used in stoichiometric amounts. In the synthesis of (*S*)-fluoxetine, one of the enantiomers of antidepressant Prozac, the crucial step, the reduction of β-chloroketone **9** to (*S*)-alcohol **10** was achieved with the use of diisopinocampheyl chloroborane (Scheme 7).

Subsequent substitution reactions proceeded with no loss of enantiomeric purity (10).

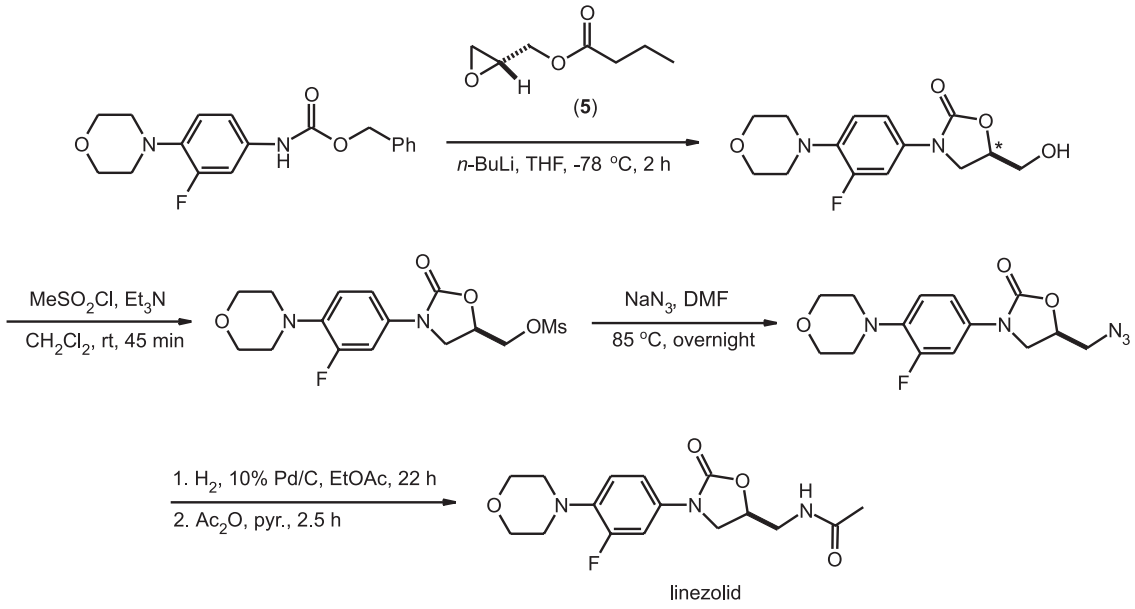
Chiral boranes, introduced by H. C. Brown and coworkers, are successfully used for asymmetric hydroboration-oxidation reactions to give chiral alcohols. Hoffmann-La Roche synthesis of anti-obesity drug orlistat is based on this reaction to introduce the first two centers of chirality in cyclopentanol derivative **11**. The use of cyclic substrate (cyclopentadiene) allows to control efficiently the selectivity of further steps of the synthesis. Thus, both the ring-opening reaction of epoxide **12** and Bayer-Villiger oxidation of ketone **13** were carried out in a highly regioselective manner (Scheme 8) (11, 12).



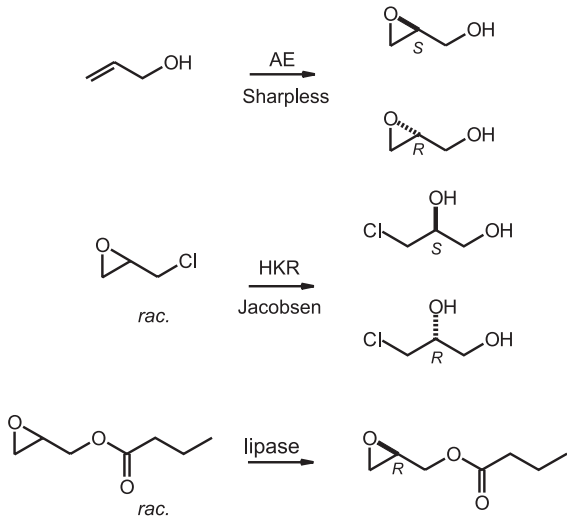
Scheme 2. Chiral pool synthesis of imipenem.



Scheme 3. Diastereoselective synthesis: tadalafil (Cialis®).



Scheme 4. Diastereoselective synthesis of linezolid.



Scheme 5. Glycidols and epichlorohydrins from chiral pool.

It is of interest to note that lactone **14** contains all the chiral carbon atoms present in the final product orlistat.

The Merck synthesis of efavirenz, a reverse transcriptase inhibitor for the treatment of AIDS, uses a highly enantioselective addition of complex chiral organozinc reagent **16** (having chiral amino alcohol ligand **15**, derived from ephedrine) to trifluoromethyl ketone **17** (Scheme 9) (2).

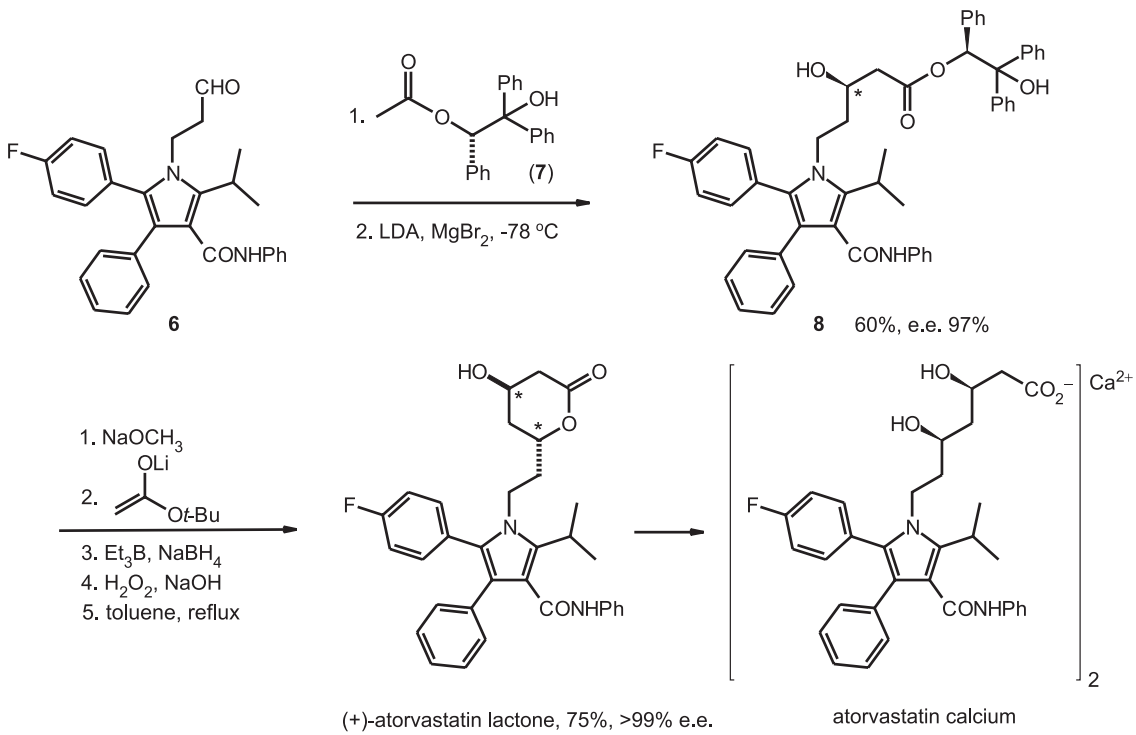
Enantioselective catalysis with metal complexes

Research in enantioselective catalysis has been fueled in the past quarter of century by the anticipated applications in fine chemicals industry. Numerous enantioselective catalytic reactions have been developed, some of the most effective and frequently applied are listed in Table 3.

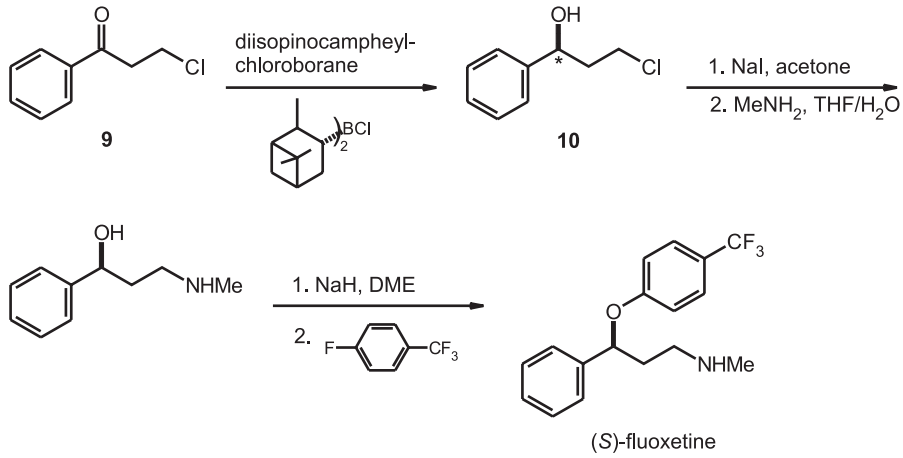
The diverse enantioselective reactions provide ways to the formation of a variety of new bonds by the addition processes (C-H, C-C, C-O, C-N, C-S, S=O) and, accordingly, new chiral centers. Enantioselective catalysis with chiral metal complexes (MC) arguably embraces the largest variety of reactions, with asymmetric hydrogenation and epoxidation best known for applications to bulk industrial processes. On the other hand, biocatalysis (BC) with the use of enzymes or whole-cell preparations is strongly gaining importance for industrial applications. Organocatalysis (OC) is an emerging alternative for metal complex catalysis, having the advantage of environmentally friendly metal-free processes. Because of their versatility and efficiency, some of the ligands/metal complexes have been singled out as privileged ones, Figure 1 (13).

Only two of the ligands listed in Fig. 1 are derived from naturally occurring compounds: TADDOLates (from tartaric acid) and cinchona alkaloid derivatives, other are synthetic. Nearly all ligands (except the monomeric cinchona alkaloid derivatives) have C_2 symmetry; this feature apparently enhances enantioselective action of their metal complexes. Esters of tartaric acid are excellent ligands in titanium(IV) catalyzed asymmetric epoxidation (AE) of allylic alcohols according to Sharpless. This process was applied to the synthesis of enantiomers of fluoxetine, using D or L enantiomers of diisopropyltartrate as ligands (Scheme 10) (14).

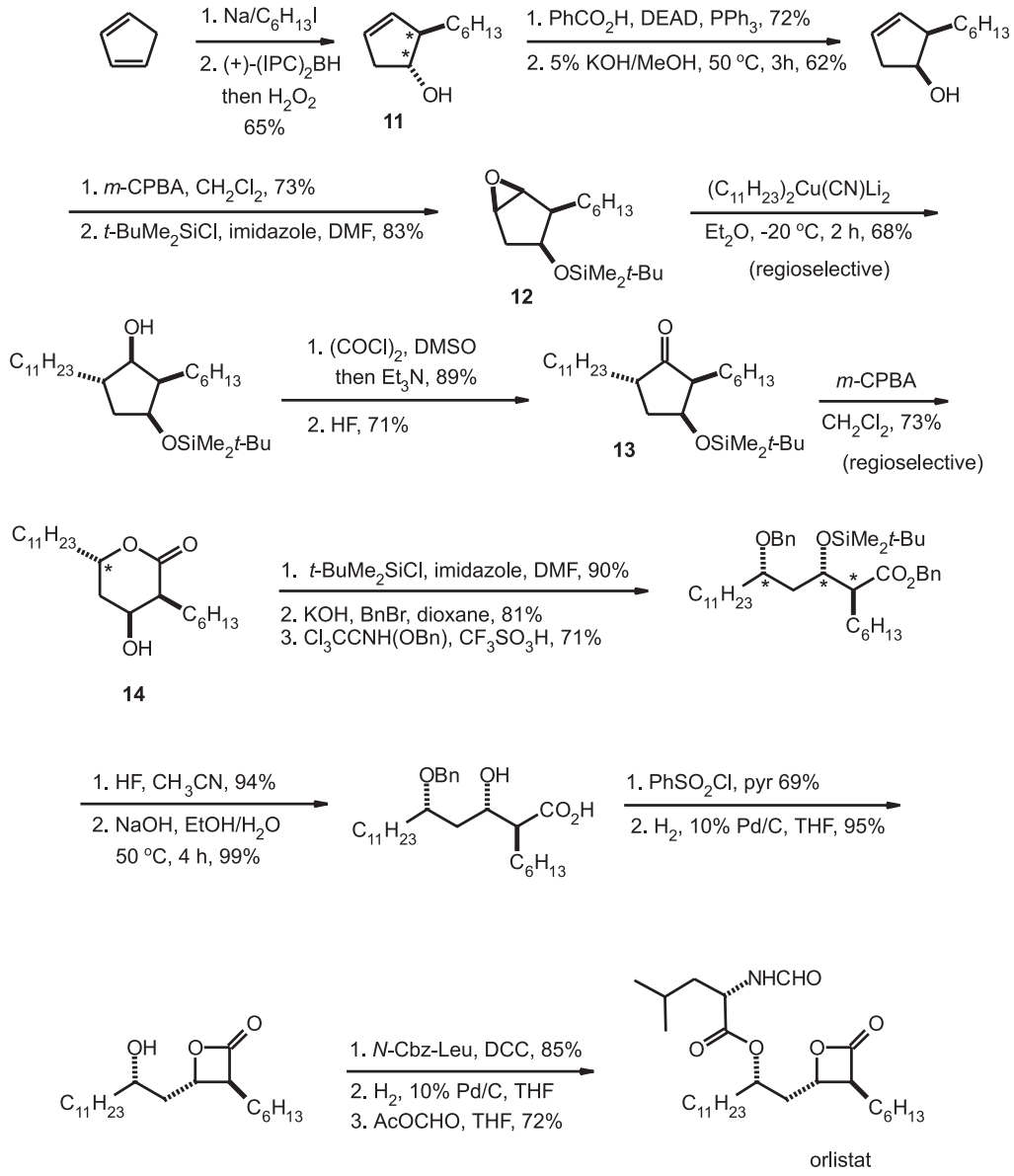
Enantioselective catalytic reduction with oxazaborolidines as catalysts, developed by H. C. Brown, is



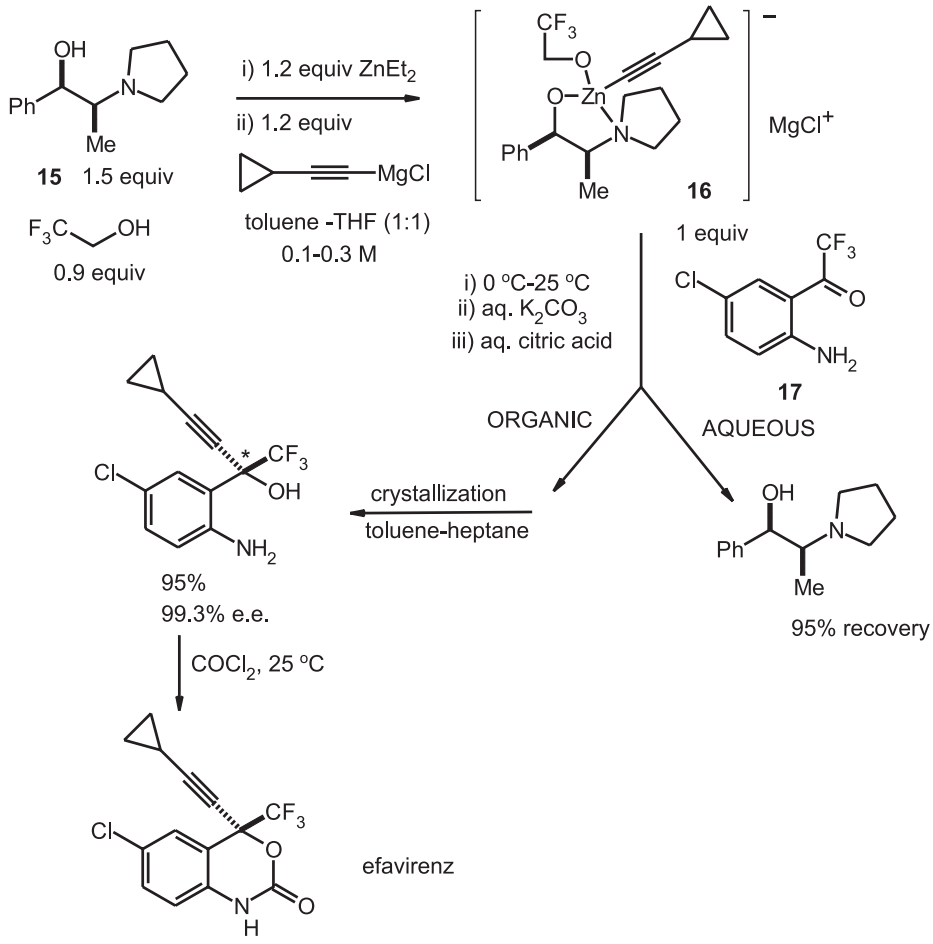
Scheme 6. Chiral auxiliary mediated diastereoselective aldol reaction: synthesis of (+)-atorvastatin lactone.



Scheme 7. Asymmetric reduction with chiral reagent: synthesis of (*S*)-fluoxetine.



Scheme 8. Asymmetric hydroboration: synthesis of orlistat.



Scheme 9. Chiral organozinc reagent: efavirenz synthesis.

Table 3. Some effective enantioselective catalytic reactions.

Process		Bond formation	Catalyst[a]
AH	asymmetric hydrogenation	C-H	MC
AR	asymmetric reduction	C-H	MB,BC
AE	asymmetric epoxidation	C-O	MC
AD	asymmetric dihydroxylation	C-O	MC
AA	asymmetric aldol reaction	C-C	MC, OC, BC
ADA	asymmetric Diels-Alder	C-C	MC, OC
ARO	asymmetric ring opening (epoxides)	C-O, C-N, C-C	MC, OC
ACA	asymmetric conjugate addition	C-C, C-N, C-S	MC, OC
HKR	hydrolytic kinetic resolution (esters, amides)	O-H	BC
OKR	oxidative kinetic resolution (sulfides)	S=O	MB, BC

[a] MC – metal complex, OC – organocatalyst, BC – biocatalyst

a convenient route for conversion of ketones to chiral alcohols. Broad range of ketones can be effectively reduced by this simple process. Examples of such syntheses are shown in Scheme 11 (15).

An example of enantioselective (and chemoselective) catalytic reduction of α-bromoketone **18** in the synthesis of antiasthmatic (*R*)-salmeterol (GSK) is shown in Scheme 12 (16).

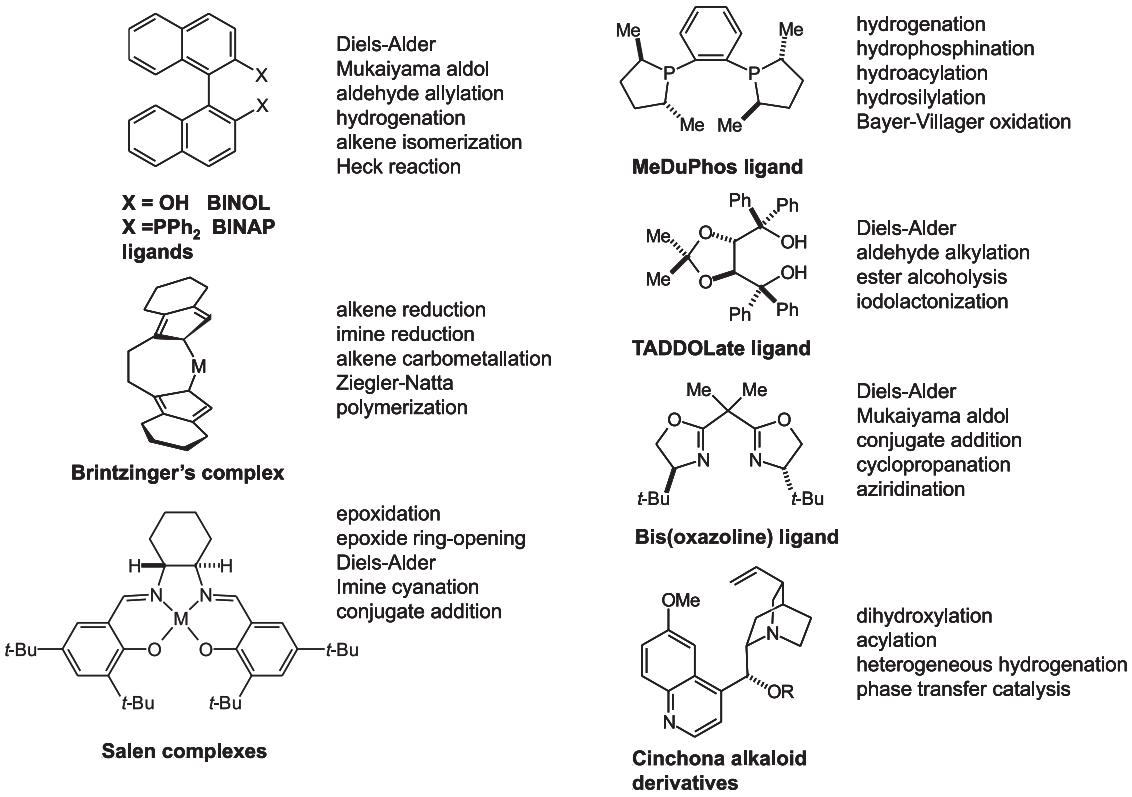
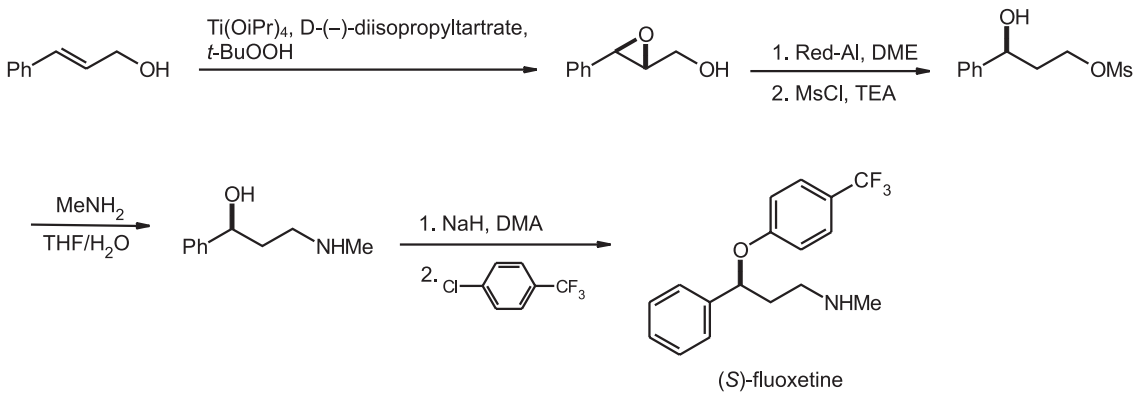


Figure 1. Privileged chiral catalysts.



Scheme 10. AE route to fluoxetine enantiomer.

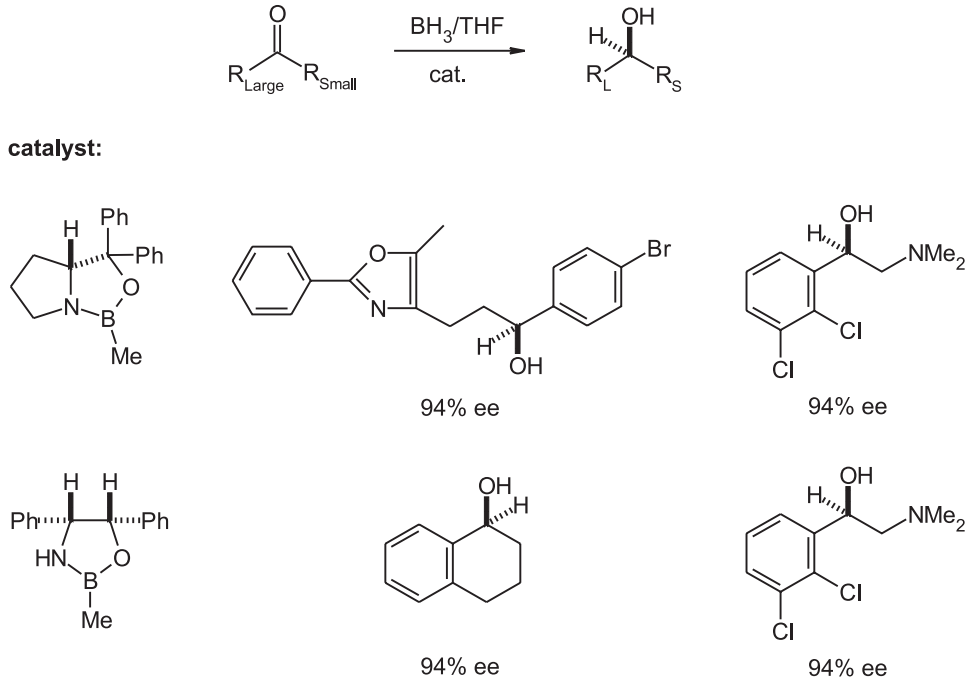
In the synthesis of antihistamine (*S*)-cetirizine (Zyrtec®, Pfizer) enantioselective and chemoselective catalytic reduction of *p*-chlorobenzophenone-$Cr(CO)_3$ complex (**19**) served as a step to introduce the center of chirality; the hydroxyl group in alcohol **20** was then substituted with inversion of configuration to yield amine **21** (Scheme 13) (17, 18).

Oxazaborolidine catalyzed reduction was applied to racemic tetralone **22**, as the method to secure the desired *S* enantiomer, from which sertraline could be obtained, according to medicinal chemistry at Pfizer (Scheme 14) (19).

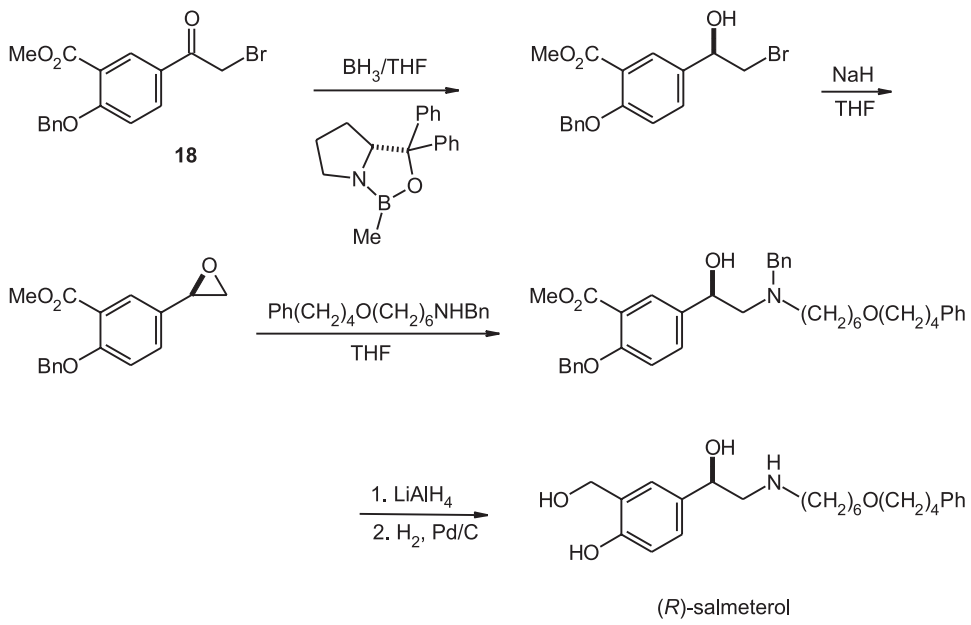
Enantioselective catalytic oxidation of a sulfide is the final step in the synthesis of H+/K+-ATPase inhibitor omeprazole, by which the chiral center is established at the sulfur atom (Scheme 15) (20).

Catalytic enantioselective hydrogenation is one of the most important methods of asymmetric synthesis. Activated carbonyl groups, such as that in ketopantolactone, are readily and effectively hydrogenated over Rh catalyst complexed with a chiral bis(diphosphine) ligand (Scheme 16).

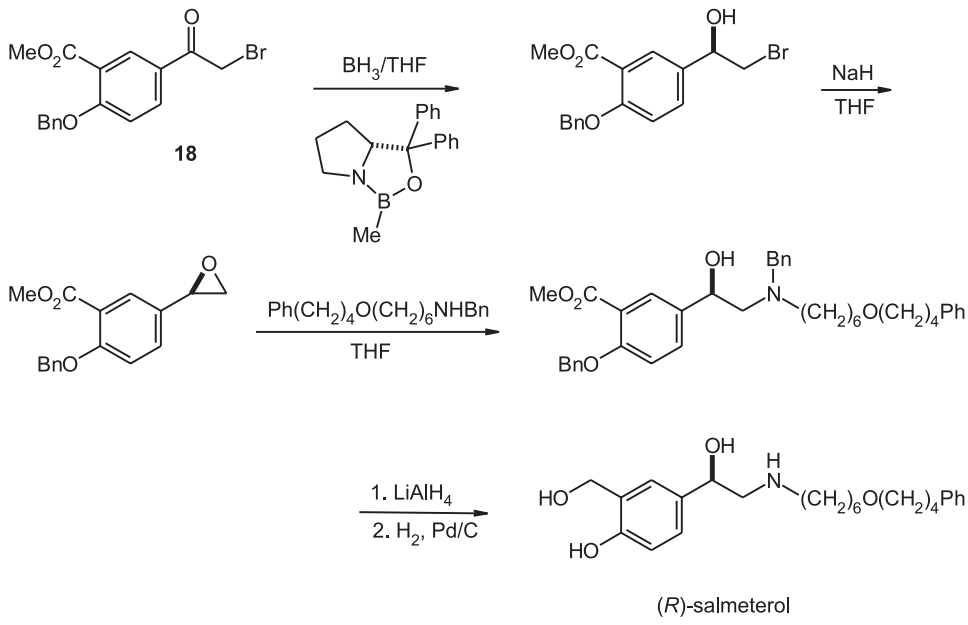
In this way, (*R*)-pantolactone is obtained in enantiomerically pure form. This can be further converted to industrially important calcium (*R*)-pantothenate (provitamin B_5) and (*R*)-panthenol. (*R*)-Pantolactone can be obtained in many different ways. The biocatalytic route (see following section) uses (*R*)-oxynitrilase as an



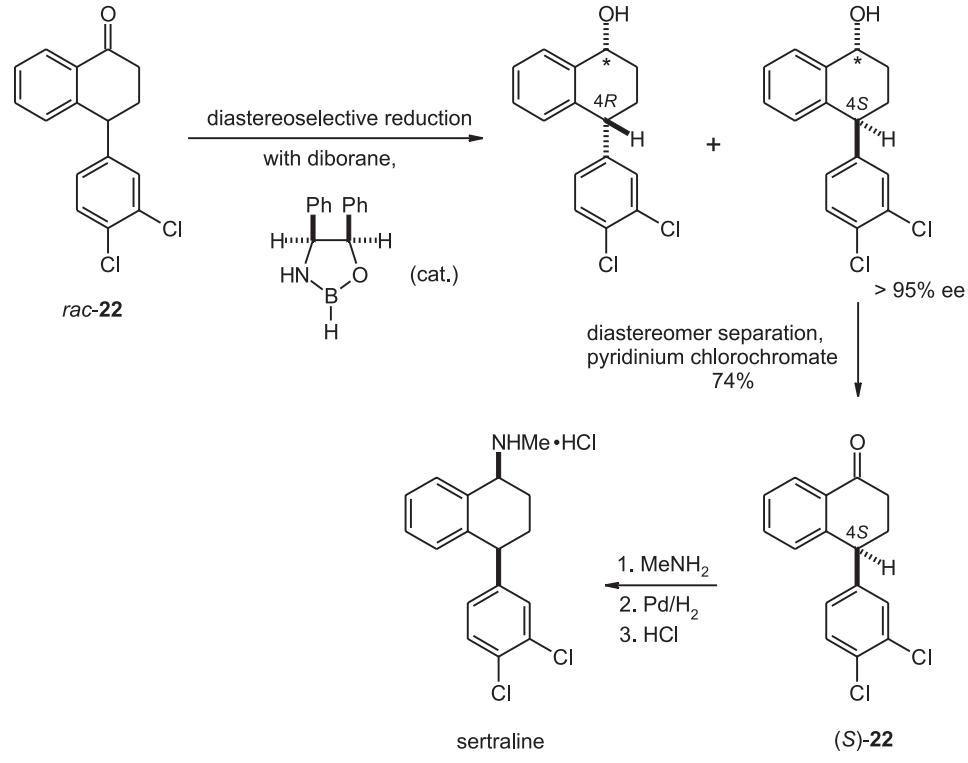
Scheme 11. Enantioselective catalysis in borane reduction.



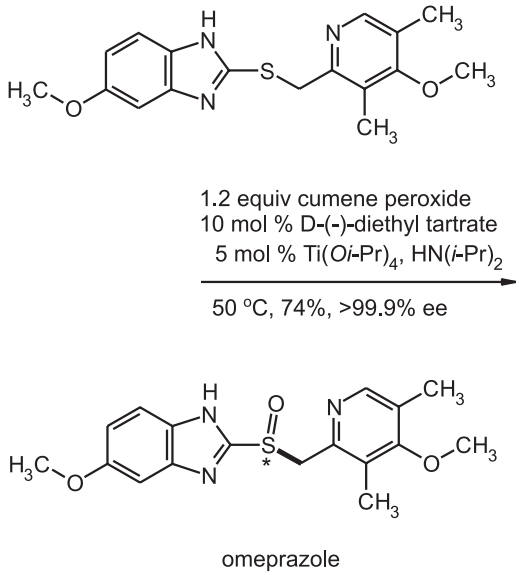
Scheme 12. Enantioselective catalytic reduction in the synthesis of (*R*)-salmeterol.



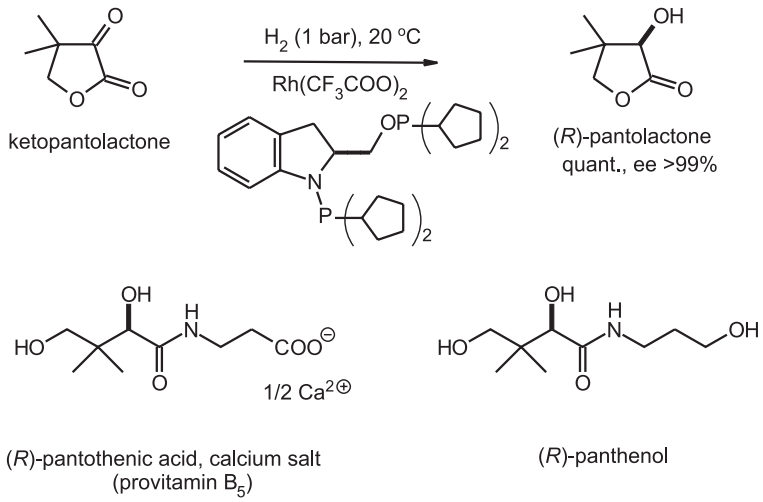
Scheme 13. Enantioselective reduction in the synthesis of (*S*)-cetirizine.



Scheme 14. Diastereoselective catalytic reduction in the synthesis of sertraline.



Scheme 15. Enantioselective oxidation of a sulfide: synthesis of omeprazole.



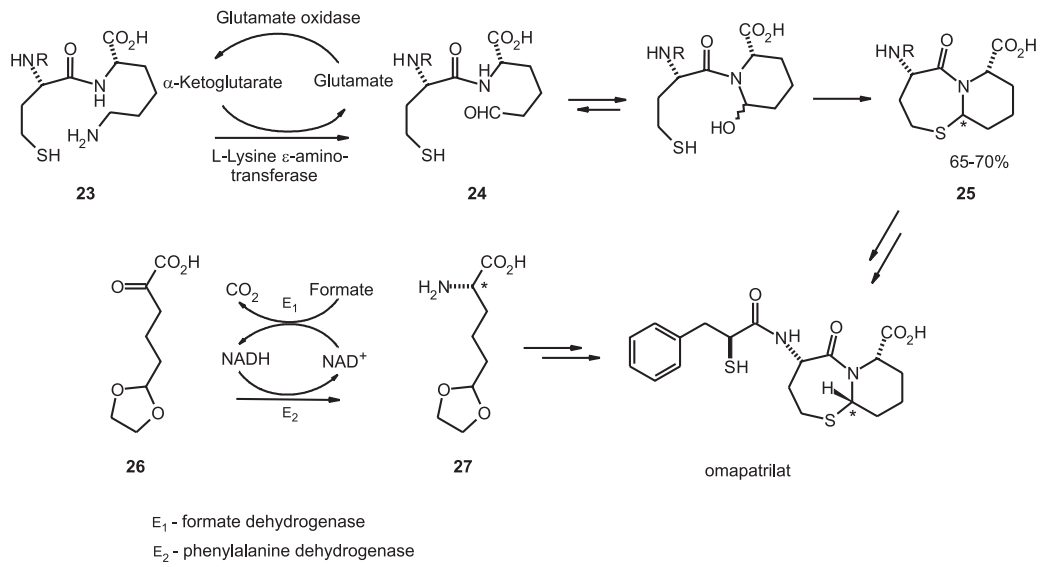
Scheme 16. Enantioselective catalytic hydrogenation: (*R*)-pantolactone.

enzyme catalyst for enantioselective addition of hydrogen cyanide to 3-alkoxy-2,2-dimethylpropionaldehyde (21).

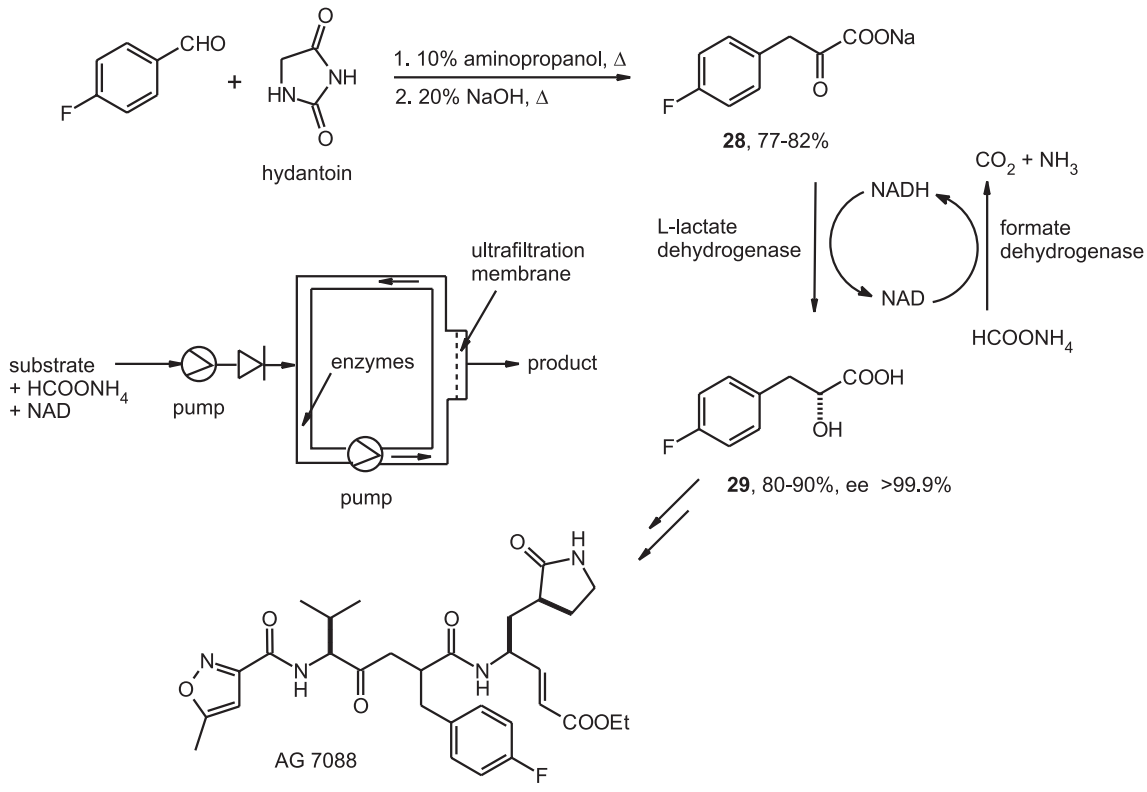
Enantioselective biocatalysis

Asymmetric catalysis with the use of isolated enzymes or whole-cell cultures has grown within past thirty years from laboratory experiment scale to thousand ton per year industrial applications. Enzyme catalyzed chemical transformations are now widely recognized as practical alternatives to traditional organic syntheses, since they usually meet stringent environmental constraints, can operate in combination with other reagents and can be engineered to broaden substrate specificity and increase enzyme activity (22). For industrial applications hydrolases and redox biocatalysts are the two categories most frequently used. For example, enantiopure alcohols as well as amines/amides are obtained from racemates by kinetic resolution with lipases in excellent yields. On the other hand, whole-cell preparations from various organisms are used at DSM to produce L-aspartic acid from fumaric acid (aspartic acid ammonia lyase from *E. coli*) or aspartame from N-protected L-aspartic acid and *rac*-phenylalanine methyl ester (thermolysine from *B. subtilis*) (23).

Whereas many biotransformations are applied to pharmaceutical industry processes, their scale is considerably smaller compared to the syntheses mentioned above and the types of biocatalyzed reactions mostly involve acylation, hydrolysis, reduction and oxidation (24). For example, at Bristol-Myers Squibb omapatrilat (vasopeptidase inhibitor) was obtained with the use of two alternative biocatalyzed steps. The engineered novel enzyme, L-lysine e-aminotransferase, oxidized dipeptide **23** to aldehyde intermediate **24** which was then cyclized to **25** with the formation of a new center of chirality. Alternatively, enantioselec-



Scheme 17. Enzymatic approaches to omapatrilat.



Scheme 18. Continuous enzymatic asymmetric reduction process.

tive reductive amination of **26** with phenylalanine dehydrogenase and recycling of the NADH cofactor provided chiral aminoacid **27** intermediate (Scheme 17) (25, 26).

An interesting case of continuous enantioselective enzymatic reduction process was reported from Pfizer. α-Keto acid salt **28** was reduced enzymatically to important α-hydroxy acid intermediate **29** for the production of AG7088 for the treatment of common cold. It used D-lactate dehydrogenase and formate dehydrogenase/ammonium formate to regenerate the costly NADH cofactor. Since both enzymes are expensive and not available in large batches a continuous membrane reactor was devised (Scheme 18). Ultrafiltration membrane unit of the reactor allowed permeation of small molecules, but not enzymes having macromolecular structure. Thus, enzymes remained inside, while the substrates were continuously fed into the reactor by a peristaltic pump. In this way, 560 g/L·day of **28** could be processed in excellent enantioselectivity (27).

Enantiomers separation by kinetic resolution

One of the most important applications of biocatalysis in fine chemicals production is for kinetic resolution of racemates. It takes the advantage of different reaction rates (k_{fast}/k_{slow}) of two enantiomers. One of the enantiomers is converted to the product, with up to 50% yield, whereas the other remains unchanged, if the reaction rates are sufficiently different. The product and the substrate are then separated by the usual physical methods (Scheme 19).

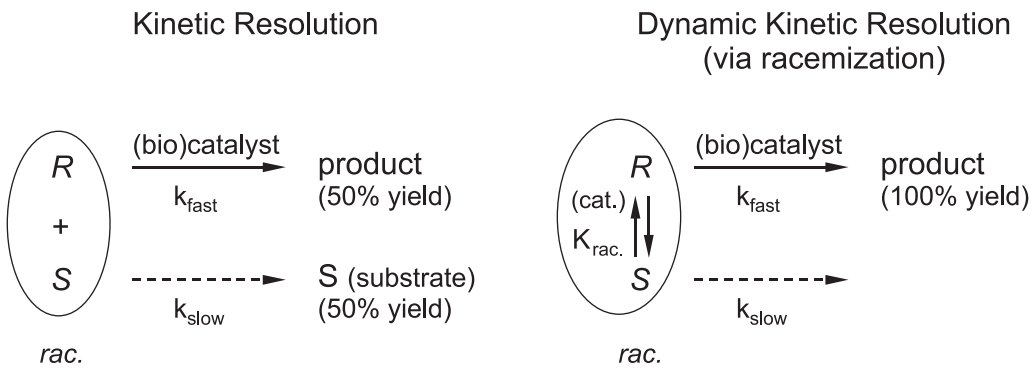
Particularly advantageous is the combination of kinetic resolution with racemization process (dynamic kinetic resolution). The racemization process can be catalyzed chemically or by a biocatalyst and the yield of the desired product can significantly exceed 50%, often approaching the theoretical level of 100%.

An example of hydrolytic kinetic resolution (HKR) performed at the final step of synthesis is provided by the preparation of enantiomerically pure enantiomer of lotrafiban (GSK) in a large scale, reliable and economic way (Scheme 20) (28).

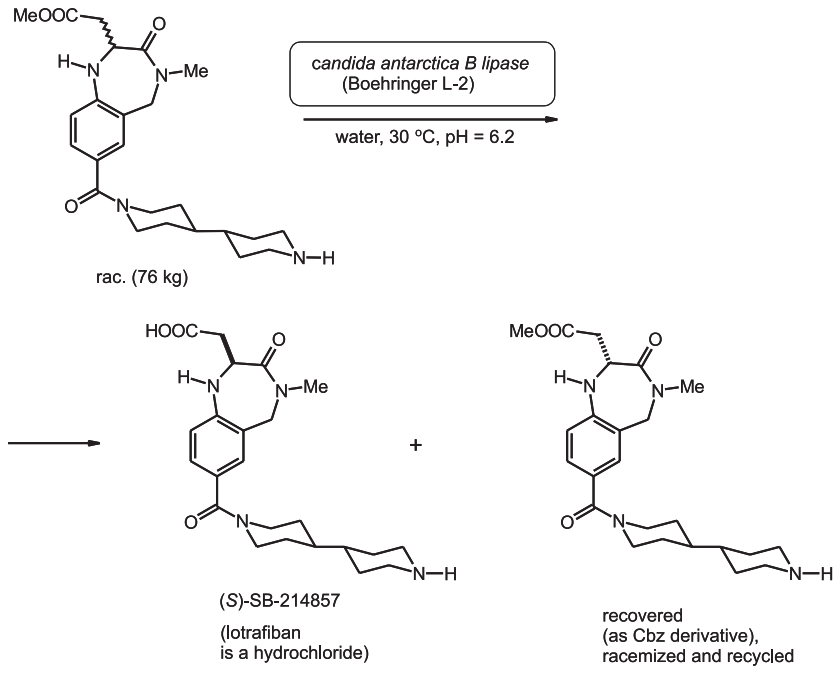
In this process the lipase catalyst is supported on a macroporous cross-linked resin for easy recovery and reuse.

Although enzyme-catalyzed processes are in general characterized by low volumetric productivity (to avoid substrate/product inhibition), some are remarkably easily performed even at high substrate concentration. An example is HKR of intermediate **30** in which one of the enantiomers undergoes C-N bond cleavage, whereas the other remains unchanged and is converted further to abacavir (Glaxo-Wellcome), the reverse transcriptase inhibitor The enzymatic HKR step is run at substrate concentration up to 500 g/L (Scheme 21) (29).

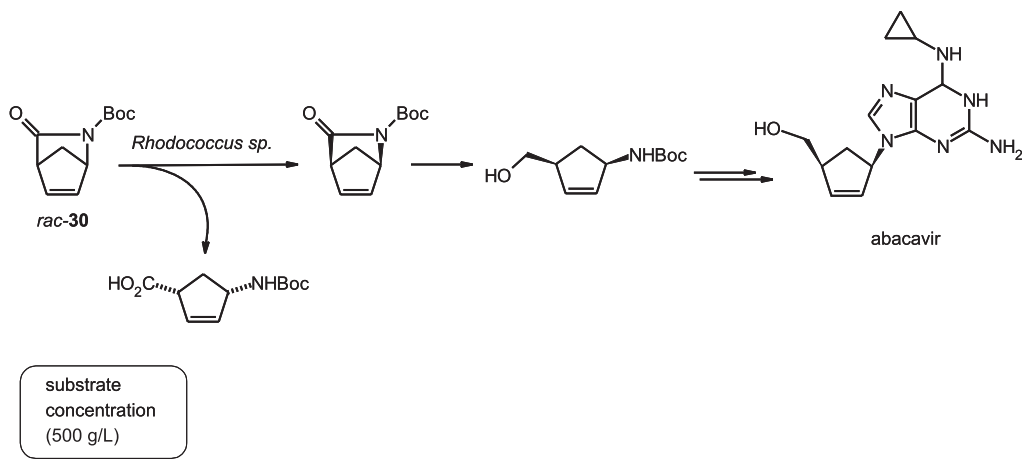
Lipases are also often used to catalyze the reverse process, i.e. the esterification of alcohols. As the acylating reagent vinyl acetate is commonly used, since it generates acetaldehyde as a byproduct which does not compete in acylation reaction. This process can obviously be applied to kinetic resolution of alcohols, such as derivatives of chlorohydrins **31** – precursors of beta blockers **32** (Scheme 22) (30, 31).



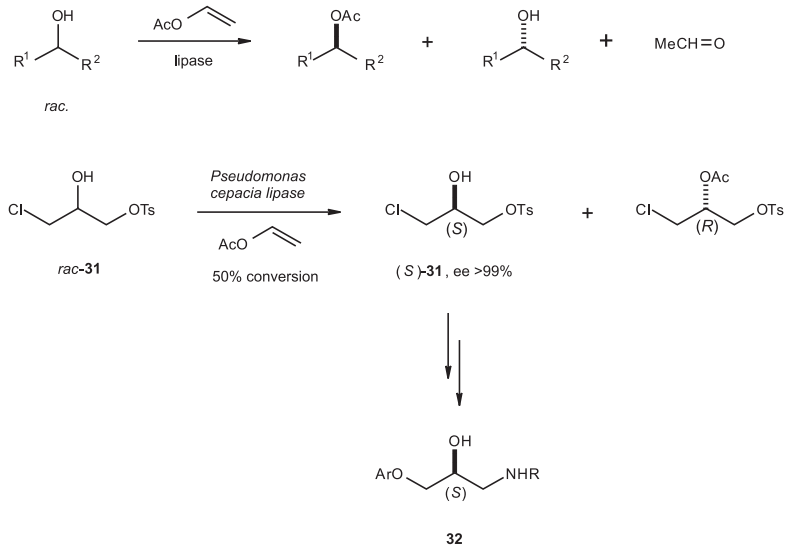
Scheme 19. Kinetic resolution and dynamic kinetic resolution.



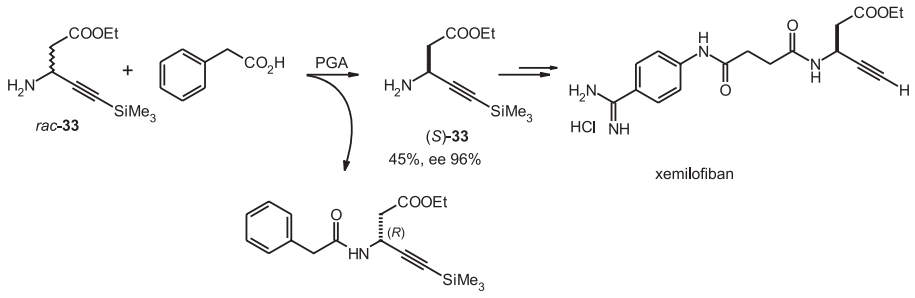
Scheme 20. Enzymatic HKR as the final step: lotrafiban (GSK).



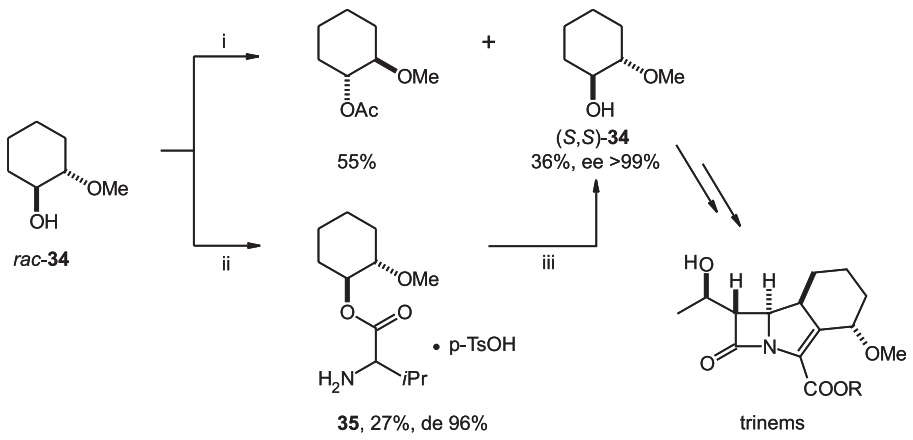
Scheme 21. HKR: synthesis of abacavir.



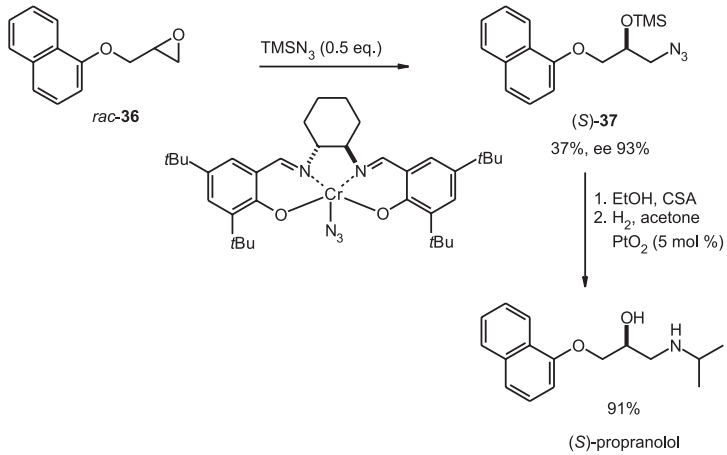
Scheme 22. Irreversible enzymatic acylation with vinyl acetate: kinetic resolution of racemic alcohols.



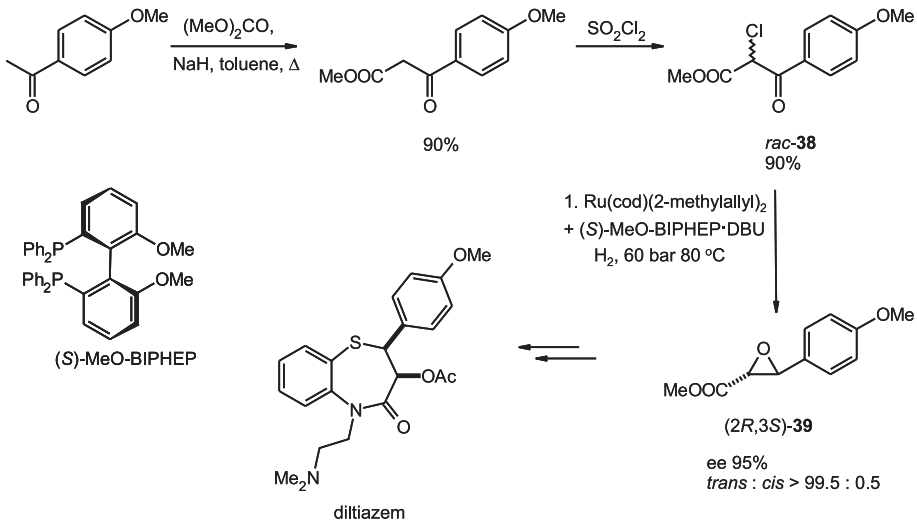
Scheme 23. Enzymatic kinetic resolution: synthesis of xemilofiban.



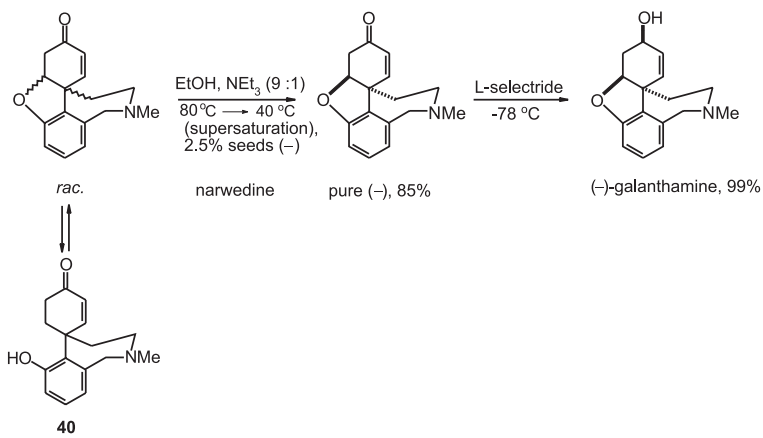
Scheme 24. Chemical vs. enzymatic kinetic resolution.



Scheme 25. Kinetic resolution of racemic epoxide: (*S*)-propranolol.



Scheme 26. Dynamic kinetic resolution in asymmetric hydrogenation: synthesis of diltiazem.



Scheme 27. Dynamic kinetic resolution by conglomerate crystallization.

The process of acylation can also be catalyzed by enzymes other than lipases. For example, racemic amine **33** could be efficiently resolved by acylation catalyzed with penicillin G acylase (PGA). Unreacted *S* enantiomer is a precursor of xemilofiban (Monsanto, Boehringer-Mannheim), an anti-platelet agent (Scheme 23) (32).

Kinetic resolution of racemic alcohols by acylation can be achieved by chemical methods. Diastereoselective acylation of racemic alcohol **34** with L-valine affords, after hydrolysis of amino acid ester **35**, *S*,*S* enantiomer of monoprotected diol **34**, with efficiency comparable to kinetic resolution catalyzed by immobilized *Candida antarctica* lipase (Scheme 24) (33).

Racemates of other types of organic compounds can also be kinetically resolved. A well-known resolution of racemic epoxides with the use of Jacobsen's chiral salen catalysts takes the advantage of differences of reaction rates with nucleophiles, such as azides. Thus, one of the enantiomers of epoxide **36** reacts faster with trimethylsilyl azide under catalysis with Cr(III)-salen to give (*S*)-**37**, a precursor of (*S*)-propranolol (Scheme 25) (34).

Dynamic kinetic resolution has been applied to the synthesis of diltiazem (Scheme 26). For this purpose epoxide intermediate (2*R*,3*S*)-**39** is obtained by asymmetric hydrogenation of racemic chloroketoester **38**. The process of reduction of the keto group is highly enantioselective, yielding (3*S*)-hydroxy derivative. This undergoes subsequently epoxide ring closure to give the desired *trans*-epoxide **39**. The last step is highly diastereoselective (*trans* : *cis* > 99.5 : 0.5) since the configuration at C-2 is at equilibrium (35).

Dynamic kinetic resolution principle can also be applied to the process of enantiomers separation by crystallization. For example, superseded solution of *rac*-narwedine in ethanol-triethylamine, when seeded with (-)-enantiomer, affords on slow cooling crystals of pure (-)-enantiomer in high yield. This is because triethylamine catalyzes ring-opening of dihydrofuran ring to give achiral cyclohexadienone species **40** in equilibrium. (-)-Narwedine can be reduced to (-)-galanthamine, an acetylcholinesterase inhibitor for treatment of Alzheimer's disease (Scheme 27) (36).

Enantioselective desymmetrization – the "meso trick"

When rates of reaction of two enantiotopic groups with chiral reagent/catalyst are sufficiently different, the reaction can be used for converting achiral, *meso* type molecule into the chiral one. Although there is no limit to the type of chiral reagent or catalyst used, in many practical applications enzymes appear most effective (37). Pig liver esterase (PLE) was found very effective in enantioselective hydrolysis of the ester groups in *meso* diesters. The reaction can in principle afford one enantiomer of the monoester product which makes it an attractive tool for large-scale synthesis. Some examples of applications for drug synthesis are given in Scheme 28.

The examples include antibiotics (+)-negamycin and (+)-thienamycin (38), (+)-biotin (vitamin H) (39) and antidepressant paroxetine (GSK) (40).

Although esterases work efficiently in water medium, the use of organic solvents is in many cases advantageous due to better solubility of substrates in such media and easy separation of the enzyme catalyst by filtration. For example, enzymatic desymmetrization of a diol by acetylation with vinyl acetate in acetonitrile, catalyzed by Novozym 435 (*Candida antarctica lipase*) was used to introduce the center of chirality in the synthesis of antifungal agent SCH51048 (41).

Enantiomers separation by crystallization and by chromatography

Crystallization and chromatography are the two methods most frequently used to physically separate organic compounds, includind stereoisomers. One of the oldest and still popular methods to separate enantiomers is crystallization of their salts with chiral counterparts – either chiral amines or chiral acids. There are countless examples of applications of such separations – both on laboratory and on industrial scale. Among chiral acids most frequently used are tartaric acid (42), its *O*,*O'*-dibenzoyl derivative (43) as well as camphorsulfonic acid. An example of the application of the latter for the separation of (+)-enantiomer of anti-thrombotic agent clopidogrel is shown in Scheme 29 (44).

For industrial applications continuous processes for enantiomers separation are preferred, in either crystallization or chromatography. For example, (*S*)-methyldopa is manufactured at Merck by the process shown in Scheme 30 (2).

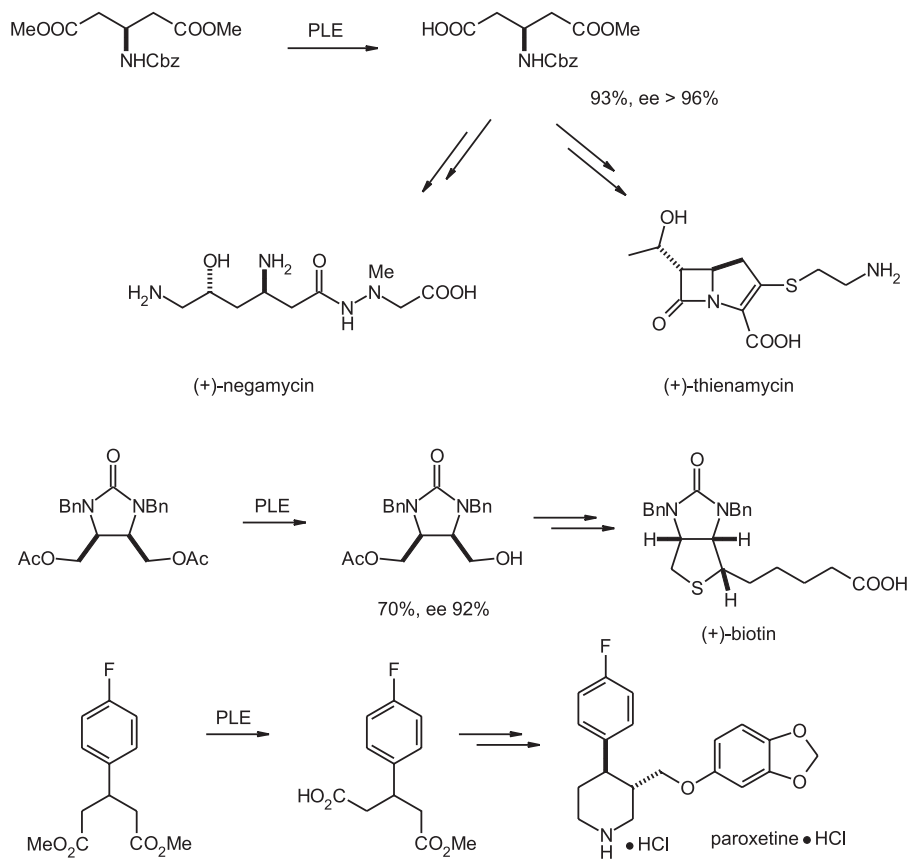
Racemic N-acetylated aminonitrile **41** is resolved by seeding with crystals of both enantiomers, in separate but connected crystallizers. This separation takes the advantage of the racemate crystallizing as conglomerate (i.e. each crystal is formed by molecules of just one enantiomer). The desired *S* enantiomer is hydrolyzed to methyldopa, whereas *R* enantiomer is racemized by the action of cyanide ion and recycled in a continuous process.

Continuous chromatography on chiral stationary phase in six columns connected to form a closed system was used to separate enantiomers of tetralone **22** in the synthesis of sertraline. Details of the process are given in publication (19). Remarkable is high enantiomeric purity of the (4*S*)-tetralone raffinate (99.7%) and high productivity of the technique (371 kg of enantiomer per year/kg of chiral stationary phase). In addition, the undesired 4*R* enantiomer can be racemized and recycled.

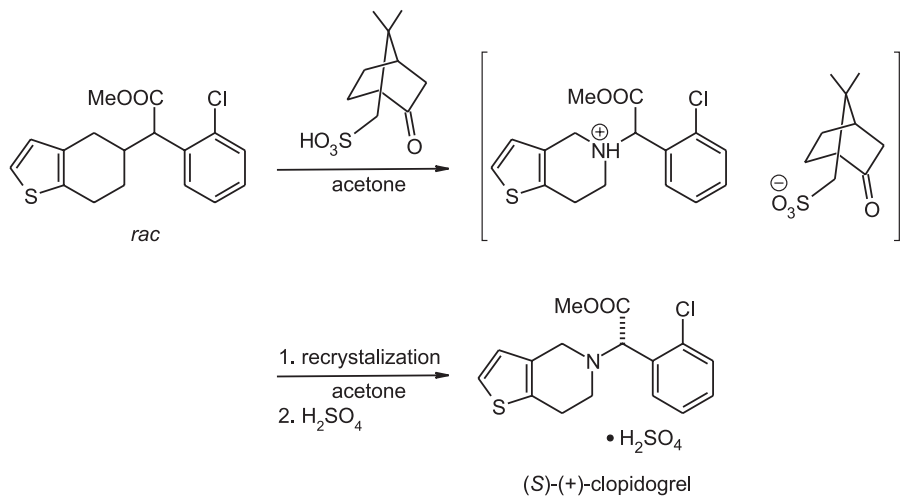
At the end it should be added that other techniques like simulated moving-bed chromatography (SMBC) are increasingly finding applications in separation of enantiomers (45).

CONCLUSIONS

Organic chemistry has provided numerous methods for multiplication of chirality, either through enantioselective or diastereoselective synthesis. Likewise, sorting of chirality through enantiomers separation by various techniques (kinetic resolution, crystallization, chromatography) has been developed to a high degree of efficiency. Asymmetric catalysis with the use of metal complexes, enzymes or organocatalysts is now of particular interest, with high expectations to meet stringent economical and environmental criteria. Drug synthesis has made significant contributions to methodolo-

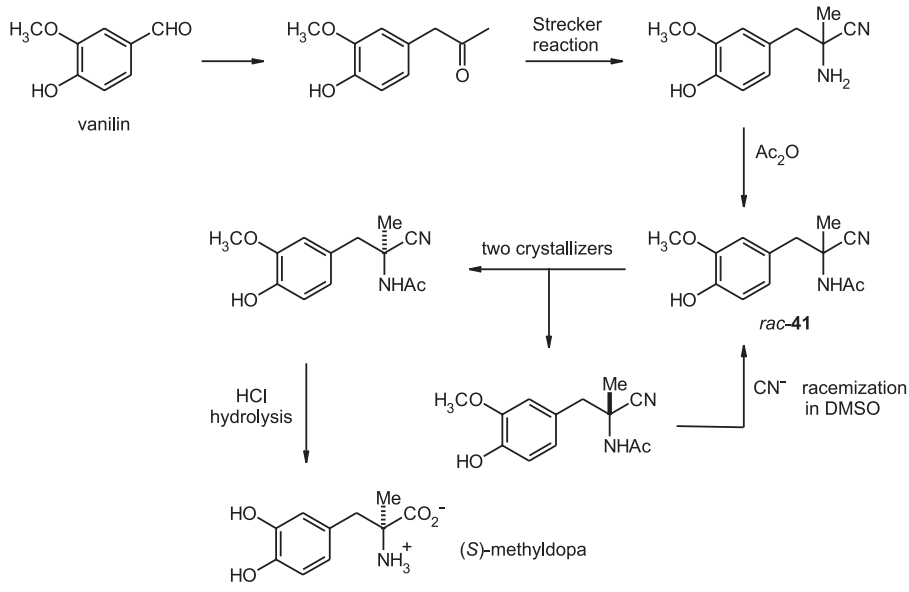


Scheme 28. Enzymatic enantioselective desymmetrizations.



Scheme 29. Enantiomers separation by diastereoisomers crystallization: (+)-clopidogrel.

gy of synthesis and, on the other hand, has benefited enormously from the advances of synthetic methodology. A general conclusion which emerges from this review is that no single method or technique has dominates the field of chiral drugs synthesis. Indeed, it is quite often that the same synthetic target is approached by different pathways or techniques in different pharmaceutical companies. Asymmetric synthesis, despite anticipations, has not eliminated alternative approaches towards enantiomerically pure products in pharma industry. Nonetheless, the use of biocatalytic methods for asymmetric synthesis and racemate resolution is on



Scheme 30. (*S*)-Methyldopa manufacturing process with continuous resolution by crystallization.

the rise. While racemate resolution is still a useful method for pharmaceutical industry, it may be even more competitive in cases where it can be combined with dynamic racemization process of unwanted enantiomer. Finally it should be noted that demands for chiral compounds differ in various stages of drug research and development (46). In early stages of development both enantiomers in small amounts are needed, with minor consideration for cost but with ready availability in short period of time (weeks). At the production stage, when tons of just one active enantiomer are required, the choice of methods used is guided primarily by scale-up feasibility, production cost and continuous delivery.

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BIODEGRADABLE NANO- AND MICROPARTICLES AS CARRIERS OF BIOACTIVE COMPOUNDS

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Abstract: This review presents examples of polymeric carriers that may be used for design of clinically aplicable drug delivery systems. In particular, there are discussed methods suitable for protein and oligopeptide modification in a way making these molecules "invisible" for immune system of an organism into which they are introduced. Presented are methods of synthesis of block copolymers suitable for self-assembly into nanoparticles loaded with bioactive compounds. There are discussed also strategies used for obtaining microparticles loaded with bioactive compounds either from earlier synthesized polymers or from monomers, directly during polymerization.

Keywords: polymeric drug carriers, nanoparticles, microparticles, pegylation

Many bioactive compounds used as active substances of drugs are decomposed or deactivated before they reach targeted organs or tissues. Delivery of oligopeptides and proteins is particularly difficult. Oral delivery of oligopeptides and proteins is often ineffective due to their disintegration in digestive tract. Delivery by intravenous or subcutaneous injection is inconvenient, whereas delivery by inhalation often leads to allergic response. Transdermal delivery is quite ineffective due to inefficiency of transport of proteins and oligopeptides through the skin.

There are hopes that some of the mentioned above problems may be solved by protecting proteins by grafting hydrophobic and biocompatible macromolecules or by packing proteins into small particles (nano or microparticles) often equipped with shells protecting particle content from premature contact with an organism. The particles are made from polymers degradable to not toxic compounds. Chemical structure of the shell should eliminate or at least reduce induction of immunoresponse of the organism.

In this short review we will discuss most promising methods based on polymeric drug carriers.

Polymers used as drug carriers

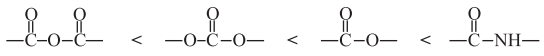
There are two basic requirements for polymers used as drug carriers introduced into an organism: First, appropriate polymers should be not harmful to an organism i.e. should be: a. not toxic, b. not cancerogenic, c. should not induce inflammatory response, d. should not induce allergic response, and e. should not affect any normal functions of patients organs.

Second, polymers used as drug carriers cannot be accumulated in patient's organism but should be metabolized to not harmful products that are eliminated by normal pathways (by renal or respiratory tracts).

Since carbon-carbon bond is very stable and cannot be easily cleaved by normal or enzymatically

enhanced hydrolysis, classical polyolefins and their derivatives cannot be used as material for carriers distributing drugs inside of an organism. Search for appropriate matrices is made among polymers with heteroatoms in the main chain: polyesters, polyanhydrides, polycarbonates, polypeptides, poly(phosphoesters) and related copolymers that are degraded to low molecular weight compounds.

Obviously, degradation rates depend on chemical structure of polymers, as well as on their crystallinity and shape of degraded specimens. Other important factors are temperature and nature of medium in which degradation takes place. Nevertheless, the general order of stability of various chemical linkages in polymers is the following:



Scheme 1. Resistance of chemical linkages towards hydrolysis.

Degradation of polymers containing mentioned above linkages results in oligomeric or small molecule diacids, hydroxyacids, diols, and amino acids that often are not toxic, not cancerogenic, not immunogenic, and induce relatively low inflammatory response. Their metabolites usually could be removed by pulmonary and renal pathways.

In some instances there may be used also not degradable water soluble polymers but they molecular weight should not exceed 69 000 (i.e., molecular weight of human serum albumin) and preferentially should be lower than 40 000 to allow for glomerular filtration.

Properties of discussed above polymers make them interesting as candidates for drug carriers (1).

Importance of size of drug carriers

Size of drug carriers is an important parameter determining their distribution in an organism. Simply, particles that are too large cannot be transported through some blood vessels. Thus, proper size adjustment may enhance localization of drug carrying particles in particular tissues and organs. Some examples are given in Table 1.

From Table 1 it follows that for controlled delivery one should use polymeric particles with required diameters and with narrow diameter dispersity.

Pegylated oligopeptides and proteins – "invisible" drug molecules

Immune system of host organism usually recognizes introduced proteins as foreign molecules and initiates a sequence of events leading to their decomposition and elimination. Thus, proteins and other proteinaceous compounds are quickly cleared from the organism. The mentioned above undesired effect may be significantly reduced by decorating proteins with biocompatible and not immunogenic synthetic macromolecules assembled into protective shells. The protective shells screen proteins from contact with antibodies of the host organism and thus make them "invisible" to immune system of an organism.

Protective shells are usually formed by grafting poly(ethylene glycol) chains onto proteins. The common name coined for these processes is *pegylation* (3, 4).

Poly(thylene glycols) used for grafting onto proteins should be equipped with appropriate chemical groups reactive usually toward amine (cysteine, histidine, lysine, serine, and tyrosine) or, in some processes, toward thiol (cysteine) polymer groups. Below are given examples of poly(ethylene glycols) used for pegylation. First syntheses were carried on using poly(ethylene glycols) activated with trichlorotriazine (see Scheme 2) (5).

Scheme 3 illustrates pegylation resulting in proteins with poly(ethylene glycol) chains linked via urethane linkages.

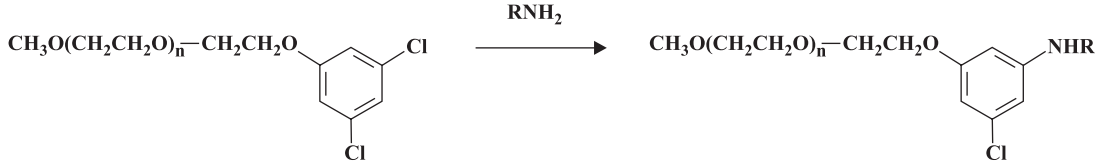
There was developed also a method for synthesis of poly(ethylene glycol) with acetal end-groups (6). Acid treatment resulted in conversion of acetal end-groups into aldehyde groups. Molecules of poly(ethylene glycol) with aldehyde groups can be bound onto protein with formation od Schiff base linkages (6, 7). The latter could be reduced to stable secondary amino groups (3). Corresponding reactions are shown in Scheme 4.

It has been found that proteins can be conveniently pegylated also with poly(ethylene glycols) with carbonylimidazole (8) and succinimidyl succinate (9) end-groups. Reactions responsible for the mentioned above pegylations are shown in Scheme 5. It is worth noting that in the cited above papers are described not only pegylation reactions but also syntheses of poly(ethylene glycols) with needed functional end-groups.

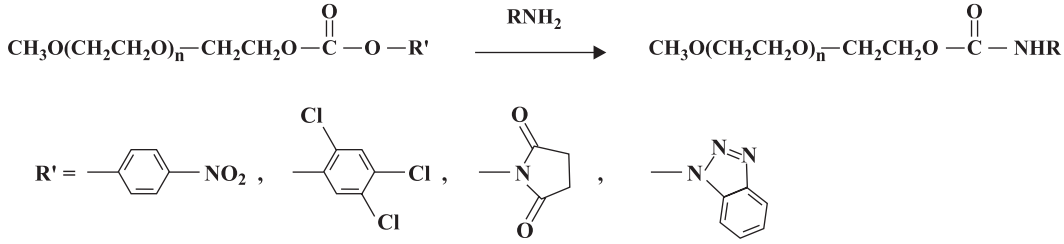
Pegylated proteins are cleared from the circulation with half-time less than 6 min. Pegylation increases this time to more than 4 h (10).

Nanoparticles

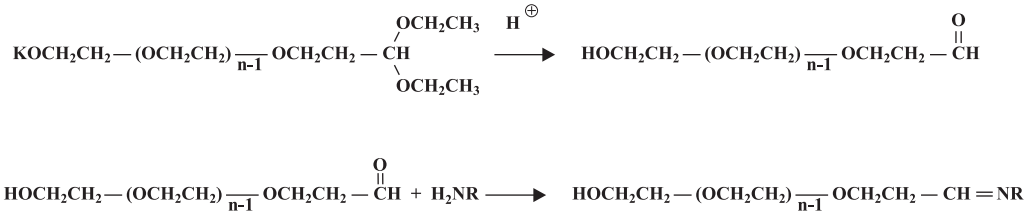
Nanoparticles (particles with diameters below 100 nm) were obtained from block copolymers with hydrophobic, biodegradable blocks (e.g. polyester blocks) and hydrophilic biocompatible blocks (e.g.



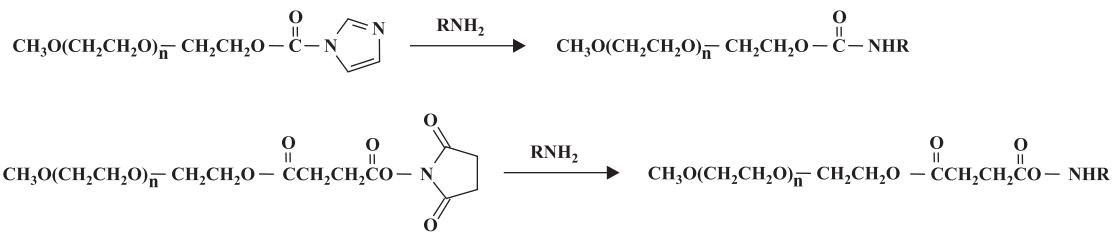
Scheme 2. Pegylation of proteins by reaction of protein amino groups (RNH_2) with poly(ethylene glycol) activated with trichlorotriazine.



Scheme 3. Pegylation by using poly(ethylene glycol) derivatives with labile acyl-oxygen bond in -C(O)-OR' group, RNH_2 denotes protein.



Scheme 4. Pegylation of proteins with poly(ethylene glycol) equipped with acetal end-groups.



Scheme 5. Pegylation of proteins with poly(ethylene glycols) with carbonylimidazole and succinimidyl succinate end groups.

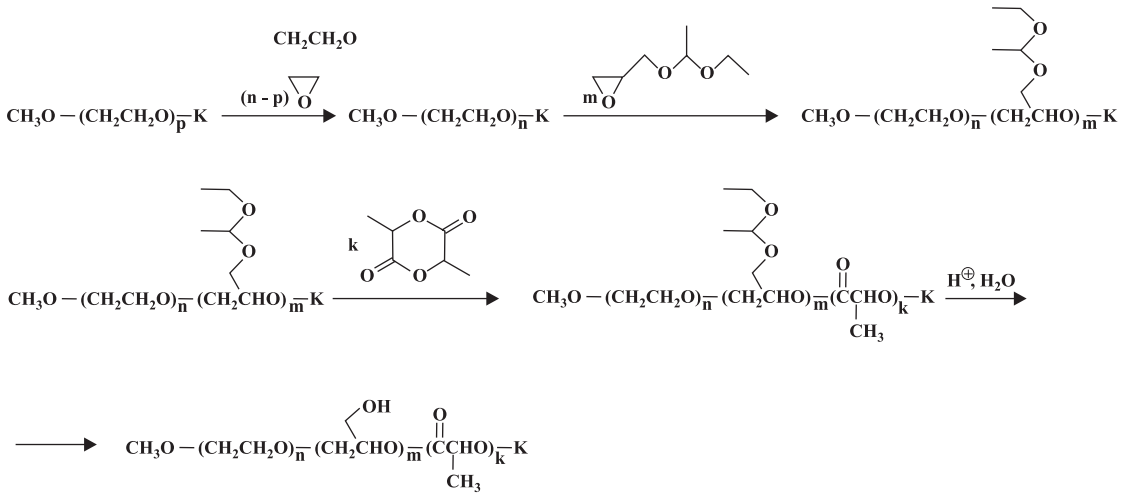
poly(ethylene oxide) or polyglycidol). The mentioned above hydrophilic blocks are not degradable. Thus, it is important to carry on syntheses in such a way that molecular weight of these blocks would not exceed 40000, needed for their removal by renal pathways.

Nanopartilces from hydrophobic-hydrophilic block copolymers may be obtained either by spontaneous self-assembly above critical aggregation concentration (CAC) (11-15) or by dialysis of copolymer solutions in organic solvents (miscible with water) against water (16-21).

Polyester-*b*-poly(ethylene oxide) block copolymers can be obtained by anionic or pseudoanionic ring-opening polymerization of ethylene oxide with subsequent (after full conversion of ethylene oxide) polymerization of cyclic esters (e.g. lactides or ε-caprolactone) (e.g. as it was described in Ref (17)).

Much more complex is synthesis of copolymers with polyglycidol blocks. These copolymers should be obtained by polymerization of derivatives of glycidol with blocked hydroxyl groups, otherwise, due to transfer reactions (involving hydroxyls) polymerization would yield branched, not linear molecules. Synthesis of poly(L,L-lactide)-*b*-polyglycidol-*b*-poly(ethylene oxide) copolymer, illustrated in Scheme 6 is an example (13).

Polyester-*b*-polyether block copolymers equilibrated in water yielded nanoparticles with diameters below 100 nm, providing copolymer concentration was above its CAC. For example, equilibration of poly(L,L-lactide)-*b*-polyglycidol-*b*-poly(ethylene oxide) block copolymer with M_n of poly(L,L-lactide), polyglycidol and poly(ethylene oxide) blocks equal 3800, 500, and 2800, respectively, dissolved in water (copolymer concentration in the range from 0.12 g/L (CAC) to 1.0 g/L; limit of solubility) and carried on at room temperature for 24 h, yielded nanoparticles with number average diameter D_n equal 27 nm and with diameter dispersity (D_w/D_n, where D_w denotes weight average diameter) equal 1.08 (13).



Scheme 6. Synthesis of poly(L,L-lactide)-*b*-polyglycidol-*b*-poly(ethylene oxide) block copolymer.

Nanoparticles formed by dialysis were usually obtained from polymers insoluble or only slightly soluble in water. Their diameters were larger than diameters of nanoparticles obtained by self-aggregation of copolymer macromolecules. For example, dialysis of triblock copolymer, poly(trimethylene carbonate)-*b*-poly(ethylene oxide)-*b*-poly(trimethylene carbonate) (M_n of poly(ethylene oxide) block 6000, wt fraction of poly(trimethylene carbonate) equal 70%) against water gave nanoparticles with diameter 77 ± 2 nm (21). Dialysis of solution of poly(L,L-lactide)-*b*-polyethylene oxide)-glucose (copolymer end-capped with glucose moiety; DP_n of poly(L,L-lactide and poly(ethylene oxide) blocks equal 48 and 54, respectively) in N,N-dimethylformamide against water yielded nanoparticles with diameter equal 42 nm (17), i.e. slightly but significantly larger than nanoparticles obtained by self-assembly of copolymer molecules in water.

Self-assembly of copolymer and drug molecules into drug-loaded nanoparticles is inefficient. Only hydrophobic compounds with limited water solubility could be encapsulated in this way. An example is encapsulation of pyrene, a model of hydrophobic bioactive compound, into poly(L,L-lactide)-b-poly(glycidol)-b-poly(ethylene oxide) nanoparticles realized by equilibration of copolymer and pyrene solution in water/1,4-dioxane mixture (1,4-dioxane constituted 10% v/v of the mixed solvent) (23). However, about 1000 fold excess of copolymer was needed for 90 % efficiency of encapsulation.

Dialysis is a much more efficient method of encapsulation. However, it could be used only in the case of compounds that are soluble in the same organic solvents (miscible with water) in which copolymers can be also dissolved. This method was used for production of nanoparticles loaded with Clonazepam (CNZ, benzodiazepine anticonvulsant) made from hexablock copolymer (see Scheme 7) with poly(γ-benzyl-L-glutamate) and poly(ethylene oxide) blocks (22).

Nanoparticles were obtained by dialysis of copolymer and CNZ solutions in N,N-dimethylacetamide, dimethylformamide, dimethylsulfoxide, 1,4-dioxane, tetrahydrofuran against water. The highest degree of loading 23.8% (wt) was obtained for dialysis from 1,4-dioxane. Diameters of nanoparticles varied from 20 ± 5 nm (dialysis from N,N-dimethylacetamide) to 150 ± 30 nm (dialysis from 1,4-dioxane). Studies of the release of CNZ into phosphate buffered saline (PBS, pH = 7.4) revealed that 80% of the release of encapsulated drug required from 2 to 7 days. This time did depend on molecular weight of poly(γ-benzyl-L-glutamate) and on the solvent from which the nanoparticles were obtained.

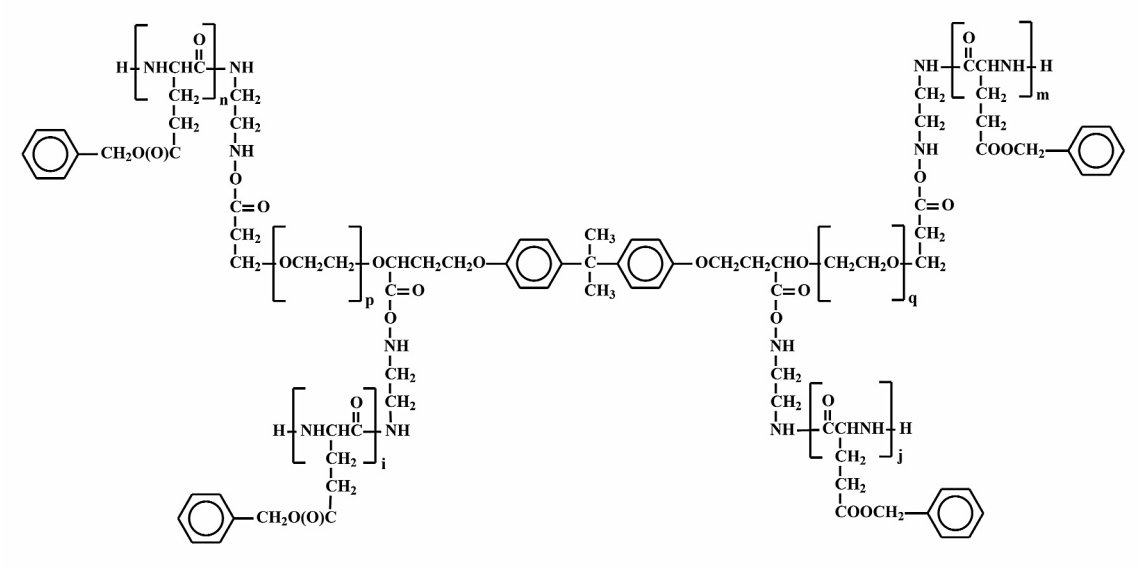
Similar method was used for encapsulation of methotrexate, an anticancer drug, into poly(trimethylene carbonate)-*b*-poly(ethylene oxide)-*b*-poly(trimethylene carbonate) nanoparticles (21). The highest achieved encapsulation efficiency was about 30% (fraction of encapsulated drug in relation to drug initially present in solution) and highest loading was 6.3% (wt fraction of methotrexate in nanoparticles).

Microparticles

Microparticles (particles with diameters in a range approximately from 100 nm to few micrometers) suitable for drug delivery can be made from homo- and copolymers that are degradable hydrolytically or enzymatically to not harmful products. There is a large variety of methods allowing for production of microparticles loaded with bioactive compounds. Selection of each particular method depends on physicochemical properties of bioactive compounds (e.g. solubility in particular solvents).

In case of drugs insoluble in water a convenient method for production of drug loaded particles is based on the emulsification-precipitation-solvent evaporation technique. This method, called oil-in-water (O/W) consists on dissolving polymer and drug in an organic solvent boiling at low temperature (water not miscible). Then the drug and polymer solution is added to water containing surface active compound. Polymer precipitates in form of particles that after evaporation of organic solvent (desirably at room temperature) are isolated by filtration or centrifugation.

Microparticles can be formed also from polymer and drug solutions in water miscible organic solvents. This method called an emulsification-precipitation-solvent extraction method differs from the one described above by the process of elimination of organic solvent (solvent extraction, not evaporation).



Scheme 7. Hexablock copolymer with poly(γ-benzyl-L-glutamate) and poly(ethylene oxide) blocks used for production of nanoparticles loaded with clonazepam (based on Ref. (22)).

A slightly more complex method is used for encapsulation of water soluble compounds. This method is called a water-in-oil-in-water (W/O/W) method and consists on emulsification of water solution of bioactive compound in polymer solution in organic solvent (not miscible with water). The water-in-oil emulsion is subsequently emulsified in water solution containing an emulsion stabilizer and organic solvent is evaporated. Below there are given selected examples of preparation of microparticles loaded with bioactive compounds by using O/W, W/O/W and emulsification-precipitation-solvent extraction methods.

The O/W method was used for preparation of poly(D,L-lactide) microspheres loaded with lomustine and progesterone (23). Diameters of these microspheres ranged from 50 to 300 μm. Drug loading was close to 20% (wt/wt). The O/W method was also used for preparation of poly(lactide-co-glycolide) microspheres loaded with bupivacaine and etidocaine (local anesthetics) (24), with oligopeptides (25), insulin (26), dexamethasone [27], and for poly(ε-caprolactone) and poly(D,L-lactide-co-glycolide) microspheres loaded with nifedipine (an antihypertensive drug) (24, 28).

The water-in-oil-in-water method was used for preparation of poly(D,L-lactide-co-glycolide) microspheres loaded with DNA (29), hepatitis B surface antigen (30), human serum albumin [31], octretoide acetate (oligopeptide) (32), and lysozyme (33).

The W/O/W method was used also for production of poly(D,L-lactide-co-glycolide) microspheres loaded with brain derived neurotropic factor, ciliary neurotropic factor, dopamine, monosialoganglioside, nerve growth factor and norepinephrine (drugs used for treatment of neurological disorders), as well as for production of microspheres loaded with 5-fluorouracil (a drug used for treatment of brain cancer) (34). By W/O/W method there were prepared also poly(L,L-lactide-*co*-glycolide) microparticles loaded with bovine serum albumin (BSA) (35).

It is worth noting that in addition to poly(D,L-lactide)s, poly(D,L-lactide-co-glycolide)s, and poly(L,L-lactide-co-glycolide)s used for preparation of microparticles by O/W and W/O/W methods also some other copolymers were found suitable for these applications. For example, there are reports on preparation of microparticles loaded with bovine serum albumin (3.4% wt/wt) from poly(D,L-lactide-co-ethyl ethylene phosphate) (36). The release of BSA from these particles had a quite complex profile. Namely, after an initial burst that did amount to 10% there was no release of protein during subsequent 45 days. Thereafter, BSA was released with a constant rate. The complete release was achieved after about 120 days.

Rate of drug release from microspheres can be tuned up by using a combination of polylactide stereoisomers for preparation of the polymer matrix. It is well known that poly(L,L-lactide) and poly(D,D-lactide) form stereocomplexes (37-41). These stereocomplexes melt at ca. 20°C higher temperatures than corresponding homopolymers and are less soluble.

Recently, there were reports on using W/O/W method for preparation of BSA loaded microparticles (ca 100 μm) from a mixture of poly(D,D-lactide)-*b*-poly(ethylene oxide)-*b*-poly(D,D-lactide) and poly(L,L-lactide)-*b*-poly(ethylene oxide)-*b*-poly(L,L-lactide) triblock copolymers (42). In these microspheres dense domains are composed from poly(L,L-lactide) and poly(D,D-lactide) stereocomplexes and water swellable domains are composed from poly(ethylene oxide). Proteins located in the latter domains should be better protected from denaturation by water-rich polymer matrix. The achieved protein loading was in a range from 2 to 4.5% wt/wt and the release was completed within about 50 days.

Very interesting microparticles were obtained by Domb et al., who found that poly(D,D-lactide) forms stereocomplexes with natural oligopeptides and proteins containing L chiral centers. In this way there were

obtained microparticles from poly(D,D-lactide) loaded with leuprolide, (LHRH; a nonapeptide analogue of luteinizing hormone releasing hormone) (43). These microparticles were obtained by mixing solutions of poly(D,D-lactide), poly(L,L-lactide), and leuprolide in acetonitrile. Mixing was carried on for 3 days at 60°C. In result of this process the microparticles with size close to 2 μm were formed. Stereocomplexation of poly(D,D-lactide and leuprolide was evidenced by presence of a melting peak at 209°C in DSC traces of this product (melting of pure poly(D,D-lactide) used for preparation of microparticles did occur at 180.3°C). The mentioned above signal was assigned to poly(D,D-lactide)-oligopeptide stereocomplex (44). *In-vivo* studies revealed that administration of poly(D,D-lactide)-leuprolide complex (1.25 mg of leuprolide per 1 kg of an animal; Wistar rats) resulted in decreased testosterone level in blood of animals to the level lower than 10 nmol/L and this level was maintained for at least five weeks.

Domb and coworkers elaborated also a method for preparation of microparticles composed from mixtures of poly(D,D-lactide), poly(L,L-lactide), poly(D,D-lactide)-*b*-poly(ethylene oxide), poly(L,L-lactide)-*b*-poly(ethylene oxide) (45). These particles were prepared by the W/O/W method. It was found that presence of poly(ethylene oxide) blocks increased rates of insulin release.

Microparticles loaded with drugs were obtained by the spray drying technique that consists on dissolving polymer and drug in water or in low boiling organic liquid (e.g. CH_2Cl_2) and then spraying the solution into a hot drying chamber. Particles are formed by solvent evaporation. Their diameters were in a range from a few to about 50 μm.

Spray drying was used for preparation of poly(D,L-lactide-co-glycolide) microspheres laded with tetracycline hydrochloride (46). Weight average diameters of these particles were from 8.6 to 22.7 μm. Drug loading was in a range from 4.9 to 8.9% wt/wt. Similar method was used for production of microspheres loaded with darbepoetin alpha (drug loading 0.5% wt/wt) (47) and etanidazole (48).

In recent years an extensive search is made for suitable carriers of nucleic acids. Such carriers are indispensable in gene therapy. Nucleic acids cannot be simply introduced by, for example, intravenous injection. In blood nucleic acids are quickly hydrolyzed by nucleases and products of their hydrolysis are quickly cleared from the blood stream. One has to remember also that cell walls are usually negatively charged and thus, the negatively charged nucleic acids cannot easily cross the wall cell barrier. The problem may be solved by packing DNAs inside of appropriate positively charged carriers. For several years studies are carried on application of viral capsids as carriers of nucleic acids and there are many reports on efficient transfection of genes transported by them. However, application of viral capsids often induces immunogenic response. Thus, an intensive search is still carried on for synthetic carriers that are not immunogenic. Until now the polymers most often used for preparation of DNA carriers included poly-L-lysine (49), poly(4-hydroxy-1-proline ester) (50), polyethylenimine (51), chitosan (52), and polyamidoamine dendrimers (53). However, some of them are cytotoxic (e.g. poly-L-lysine and polyethylenimine) or are not degradable. Thus, a search for better carriers is still needed. Recently, there were reports on synthesis of polyphosphoramidates substituted with moieties containing primary, secondary and tertiary amine groups (54, 55). Structures of these compounds are shown in Scheme 8. The polyphosphoramidates with amino groups were used for preparation of nanoparticles loaded with pRE-Luciferase plasmid and bcl-2 gene (55). Size of obtained complex particles varied from 200 nm to 1.3 μm, depending on ionic strength of the medium in which they were prepared. Diameters of particles obtained at low ionic strength were close to 200 nm. These particles were used for effective transfection of luciferase marker gene and therapeutic bcl-2 gene (55).

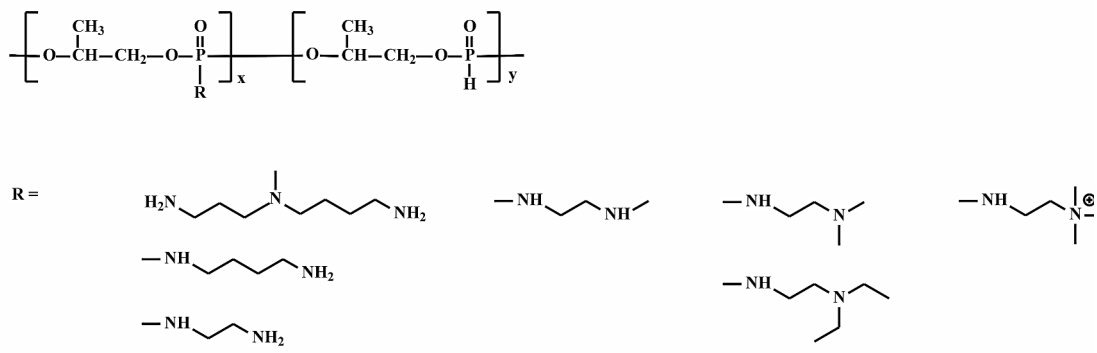
In our labioratory there was developed a method allowing for direct synthesis of poly(D,L-lactide), poly(L,L-lactide) and poly(ε-caprolactone) microspheres (56-61). Microspheres were synthesized by dispersion polymerization of lactides or ε-caprolactone initiated with tin(II) 2-ethylhexanoate (56, 57) or 2,2-dibutyl-2-stanna-1,3-dioxepane (57) (polylactides), diethylaluminumethoxide (56, 59) or sodium trimethylsilanolate (60) (poly(ε-caprolactone)). Polymerizations were carried on in heptane:1,4-dioxane mixtures (1:8 and 1:4 v/v ratio for polymerizations of ε-caprolactone and lactides, respectively) at room temperature (ε-caprolactone) or at 95°C. Poly(dodecyl acrylate)-g-poly(ε-caprolactone) was used as particle stabilizer. Synthesized polyester microspheres were transferred from organic media to water by controlled hydrolysis of polyesters in their surface layers (62).

Following strategies could be used for loading microspheres produced directly by ring-opening polymerization with bioactive compounds.

Proteins (human serum albumin and human γ-globulins) could be adsorbed onto poly(D,L-lactide) and poly(ε-caprolactone) microspheres by incubation of particles with protein solution (56). Maximal surface concentrations of adsorbed proteins (at saturation) was equal 1.0 mg/m^2 and 2.6 mg/m^2 for HSA and gG, respectively, attached onto poly(ε-caprolactone) particles and 0.9 mg/m^2 and 1.7 mg/m^2 for HSA and gG adsorbed onto poly(D,L-lactide) microspheres.

Liquid, water not miscible and polyester swelling bioactive compounds could be loaded into polylactide and poly(ε-caprolactone) microspheres by incubation of particles in ethanol/water mixture containing drug (62). For example, loading of ethyl salicylate into poly(ε-caprolactone) microspheres by the described above method yielded particles with drug content up to 37% (wt/wt).

Bioactive compounds with hydroxyl groups may function as chain transfer agents in dispersion polymerization of cyclic esters and in result could be incorporated into polymer microspheres. Poly(ε-caprolactone) microspheres loaded with N,N-bis-(hydroxyethyl)isonicotinamide were obtained according to this method (63).



Scheme 8. Polyphosphoramidates substituted with moieties with primary, secondary and tertiary amine groups – polymers used as DNA carriers.

Table 1. Diameters of polymeric particles (D) and their predominant localization in human body (based on data from Ref. (2))

Diameter of polymeric carrier	Localization in the body
D > 10 μm	Particles too large to pass capillary blood vessels of many organs. Local activity
5 μm < D < 10 μm	Particles captured in lungs
D < 5 μm	Particles captured by MPS (mononuclear phagocyte system)
1 μm < D < 3 μm	Particles captured in spleen
0.1 μm < D < 1 μm	Particles captured in liver
50 nm < D < 100 nm	Particles captured in bone marrow
D < 20 nm	Particles and giant macromolecules circulating in blood; captured in selected organs only due to specific interactions

CONCLUSIONS

Due to the limited size this short review cannot be considered as comprehensive presentation on drug delivery systems based on nano- and microparticulate polymeric drug carriers. However, we do hope that it presents the most important directions explored in last years and will be an inspiration for further studies.

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