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Reagents for (ir)reversible enzymatic acylations

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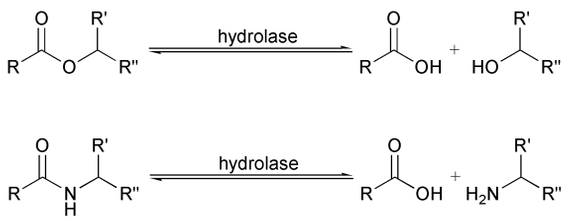
Commonly, hydrolase-catalysed reactions are used for kinetic resolutions of alcohols, amines and acids. Only a few applications in total syntheses have been described. Most of these examples are for the synthesis of amides and peptides. Here, the synthesis of all the different types of activated acids that can be applied in hydrolase-catalysed acylations is described. The application of these activated acids for both the formation of esters and amides is discussed and the different reactions are compared with the equivalent chemical reactions.



Scheme 1 Hydrolase-catalysed reactions.

1 Introduction

It is now more than one hundred years ago that Emil Fischer introduced the "Lock & Key" principle.[1] In his study of the influence of configuration on the activity of enzymes he observed their great stereoselectivity. It is there that he stated: "Um ein Bild zu gebrauchen, will ich sagen, dass Enzym und Glucosid wie Schloss und Schlüssel zu einander passen müssen, um eine chemische Wirkung auf einander ausüben zu können. Diese Vorstellung hat jedenfalls an Wahrscheinlichkeit und an Werth für die stereochemische Forschung gewonnen, nachdem die Erscheinung selbst aus dem biologischen auf das rein chemische Gebiet verlegt ist".† He leaves no doubt that he considers enzyme-catalysed reactions to be pure chemistry and of great importance to stereochemistry. It is interesting to see, to what extent Emil Fischer's assessment is correct.

In today's organic chemistry one class of enzymes is particularly well established, the hydrolases (esterases, lipases, proteases).[2,3] As the name indicates these enzymes catalyse the hydrolysis, but also the formation, of esters and amides (Scheme 1). Today, a wide variety of these enzymes is commercially available and can be used as delivered. The application of hydrolases for the formation of new C–O and C–N bonds and especially the tools developed to this end, are the focus of this article. An essential step towards utilizing the full potential of the hydrolases was their application in organic solvents.[4,5] With this approach the insoluble enzymes can not only be used for the synthesis of esters and amides, but they can also be easily removed from the reaction mixtures by filtration. 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), diisopropyl ether, toluene and hexane (which should be replaced by the less toxic heptane) have proven to be particularly versatile. They should be dry, but since some enzymes are deactivated by water-free solvents, it might be advantageous to add salt hydrates to establish and control extremely low water levels in the solvents.[6,7] The influence of water levels and the water activity (a_w) has been the subject of many investigations and it has been shown that the a_w can have a significant influence on the enzyme activity and selectivity.[8] However, there are also many examples were the amount of water present in the reaction mixture had a modest influence only.[9] In the majority of the hydrolase catalysed reactions no efforts are made to control the a_w during the reaction. Most of the powdered or immobilised enzymes show a memory effect. They tend to keep the conformation and ionisation state that they had when they were dried.[2,3,5] However, acid–base changes during the reaction can cause the ionisation state and, therefore, the conformation to change. This might have an influence on the selectivity and activity of the enzymes. In general, there is little literature precedence for conformational changes. If they occur they do not seem to have great influence on most reactions. If necessary, the acid–base conditions of enzyme-catalysed reactions in organic media can readily be controlled by solid buffers.[10,11] In summary, one can state that commercially available enzymes, with enantioselectivities that rival any transition metal catalyst, can readily catalyse reactions in organic solvents under conditions that are suitable for most undergraduate teaching laboratories. It might,

† To use a picture I would like to say, that enzyme and glycoside have to match each other like key and lock in order to potentially have a chemical effect on each other. This concept has become more likely and has gained value for the investigation for stereochemical research, now that it is part of the field of pure chemistry rather than biology.

Ulf Hanefeld was born in 1966 in Köln, Germany, and grew up in then West-Berlin and London. In 1993 he received his PhD from the Georg-August-Universität zu Göttingen, having performed the research both in Göttingen (Professor Laatsch) and Seattle (Professor Floss). After many postdoctoral years with Professor Rees at Imperial College London, UK, Professor J. Staunton in Cambridge, UK and Professor J. J. Heijnen in Delft, the Netherlands he now holds a position as lecturer at the Technische Universiteit Delft in the Netherlands. When he is not busy with enzymes or transition metal catalysts he enjoys to cycle, especially when he is going with the wind.

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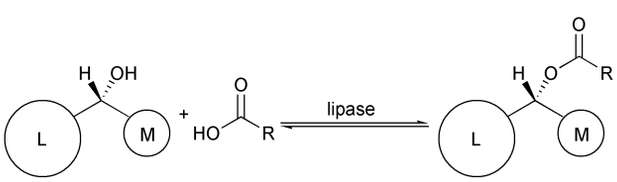
therefore, be expected that enzymes, and especially hydrolases, are part of every chemist's toolbox. This review focuses on the potential of hydrolases for the synthesis of esters and amides from alcohol/amines and activated acids, and especially on the preparation of the activated acids.

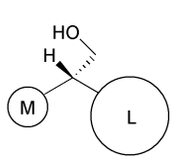
2 Enantioselectivity

When planning a synthesis of an ester or an amide one commonly starts with enantiopure compounds; *i.e.* the catalyst for the ester/amide formation does not have to show any enantioselectivity. However, since hydrolases in general are enantioselective and in some cases even almost enantiospecific, a brief description of their enantiopreferences is given. Indeed, the great value of lipases, esterases and proteases for synthesis is, in part, due to their high enantioselectivity. Especially lipases are widely used in kinetic resolutions of secondary alcohols and a model for their enantioselectivity has been established, known as the Kazlauskas rule.[12–15] This empirical rule is based on the observation that many lipases preferably catalyse the conversion of one of the enantiomers in both the synthesis and the hydrolysis reaction (Scheme 2). This stereoselectivity has been explained by the spatial arrangement of the catalytic residues on the basis of X-ray studies[16] and *via* the stereo-electronic theory.[17] In the case of chiral primary amines the rule also tends to give reliable results.[18] In this context, it is important to notice that proteases (subtilisin) commonly show the opposite enantioselectivity.[19] Enzymes for the catalytic conversions of either enantiomer of chiral secondary alcohols and chiral primary amines are, therefore, available and they tend to follow the Kazlauskas rule.[20]



Scheme 2 Kazlauskas rule: The faster reacting enantiomer of a secondary alcohol in most lipase-catalysed acylations or the faster reacting enantiomer of an ester in most lipase-catalysed hydrolyses is the enantiomer depicted. L is the largest substituent, M the medium sized substituent. In the case of subtilisin the reactions of the opposite enantiomers tend to be catalysed. The same rule applies to most primary amines.

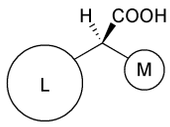
Pseudomonas cepacia lipase (PCL) catalysed conversions of primary alcohols with a chiral carbon in the β-position also follow a general rule (Scheme 3). However, it is only reliable if there are no oxygen substituents on the chiral carbon.[21] Modelling studies helped to establish the relevant binding pockets. These could then be chemically modified, thereby improving the selectivity in the case of PCL.[22]



Scheme 3 PCL preferably catalyses the acylation of the depicted enantiomer of the chiral primary alcohol; no oxygen atom should be bound to the chiral carbon, M represents the medium sized substituent, L the largest substituent.

The enantioselectivity of lipases towards chiral acids or their esters is less predictable. However, *Candida rugosa* lipase (CRL) has a stereochemical preference for one enantiomer of acids or their esters with a chiral α-carbon (Scheme 4).[23]

All these rules are based on the careful evaluation of countless experiments. The enantioselectivity is not always very



Scheme 4 Preferred enantiomer in CRL mediated hydrolysis and esterification reactions (L = large substituent; M = medium sized substituent).

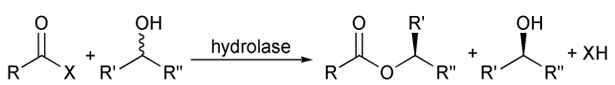
large and examples with the opposite selectivity are known. However, with these general rules in hand, it is possible to address a multitude of synthetic problems. For the detailed choice of the enzyme to be the most suitable for a particular substrate, several excellent reviews can be recommended.[2,3,24–29]

For the planning of a synthesis the hydrolase might only be envisioned as a catalyst for the gentle formation of an ester or amide bond without any stereoselectivity. Even if either the acid or the alcohol/amine has the "wrong" stereochemistry; *i.e.* violates the rules described above, this is no reason not to use a hydrolase. The enzymes show an enantiopreference: an enzyme with a low enantioselectivity will still efficiently catalyse the desired reaction, even if the "wrong" enantiomers are presented to it.

3 Enzyme-catalysed acylations

The formation of esters and amides is an equilibrium reaction (Scheme 1). The equilibrium depends on the amount of water in the reaction mixture. However, even if the reaction of acid and alcohol is performed in dry, hydrophobic solvents (hexane) the ester is not obtained in quantitative yield.[6] Several options exist to solve this problem. The simplest is of course to use a huge excess of one of the two reagents (possibly even as the solvent). As long as the substrate is readily available and high concentrations are not harmful for the enzyme this is a good solution. These two criteria are, however, hardly ever fulfilled. The removal of water from the reaction *via* (azeotropic) distillation requires conditions that are often detrimental to protein structures, causing deactivation of the enzymes. Even if the enzymes are not deactivated, higher temperatures often decrease the enantioselectivity. The two obvious methods to shift the equilibria are thus not suitable for most syntheses.

Instead of acids, activated acid derivatives can be utilised (Scheme 5). The leaving group X should be a weak nucleophile that cannot attack the ester formed. The equilibrium will therefore lie on the product side, ensuring a complete acylation of the alcohol or amine.



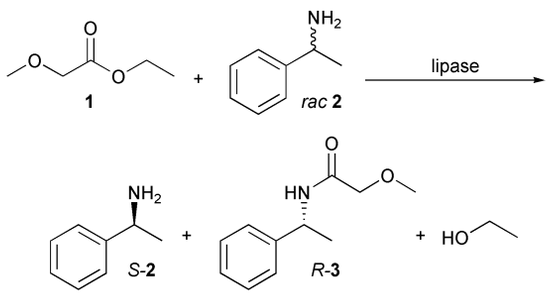
Scheme 5 Hydrolase-catalysed kinetic resolution of a secondary alcohol.

A large variety of activated acids, commonly called acyl donors, has been developed. Several excellent older reviews about the application of various commercially available acyl donors exist.[30,31] Here, the focus will be on the different types of acyl donors, their preparation and their potential for synthesis in general. All these donors can be used for the irreversible acylation of alcohols and/or amines. They are, however, generally classified as reversible, quasi-irreversible and irreversible acyl donors. The first two only act as irreversible acyl donors if the correct reaction parameters are maintained.

3.1 Reversible acyl donors

The hydrolase-catalysed formation of esters or amides from acids and alcohols or amines is a reversible reaction (Scheme 1). If an ester or a thioester is utilized as the acyl donor, the reaction remains reversible, however, compared to an amine, the alcohol released might be unreactive or the liberated thiol

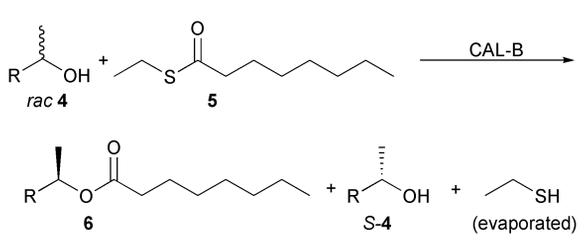
might be very volatile. Both approaches have been utilized. The electron-withdrawing effect of a methoxy group activates ethyl methoxyacetate **1** (Scheme 6).[32] In the presence of lipases **1** readily reacts with amines, while it shows no such activity in their absence. Since amines are much better nucleophiles than alcohols, the ethanol released during the reaction does not have a negative influence on the equilibrium and the products are obtained in good yields. Indeed, this reagent is now used on an industrial scale for the kinetic resolution of a variety of amines.[33]



Scheme 6 Example of an industrial, lipase-catalysed kinetic resolution of a primary amine utilising ethyl methoxyacetate **1** as the acyl donor. The lipase follows Kazlauskas rule.

Although ethyl methoxyacetate **1** is commercially available and very reactive, only a few applications outside industry have been published.[34]

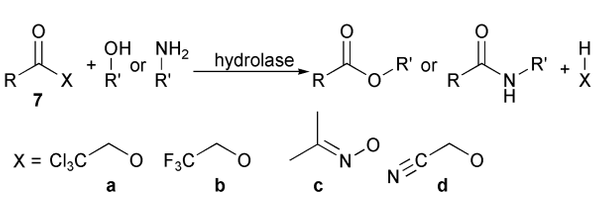
The application of thioesters as acyl donors was first reported in 1993. *S*-Ethyl thiooctanoate **5** is not only very reactive; the thioethanol released during the reaction evaporates, thus shifting the reaction equilibrium (Scheme 7). This was an improvement on an earlier system where the ester was utilized and vacuum had to be applied to the reaction mixture in order to evaporate the released ethanol.[35] Although thioesters can readily be prepared from acid chlorides or anhydrides and thiols or *via* DCC coupling directly from acids and thiols,[36] this type of acyl donor has not established itself as a general tool, possibly due to the odour of the leaving group.



Scheme 7 *Candida antarctia* lipase B (CAL-B) catalysed kinetic resolution of secondary alcohols with a thioester as an acyl donor.

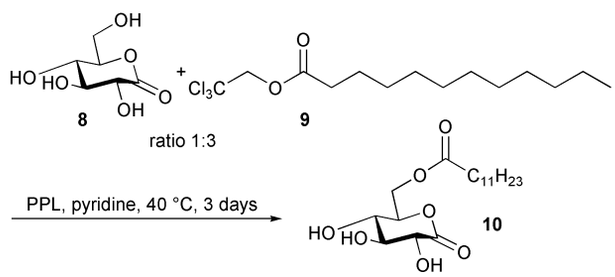
3.2 Quasi-irreversible acyl donors

In the case of the quasi-irreversible acyl donors (**7a**–**d**) the released XH (Scheme 8) is a very weak nucleophile or might even decompose slowly under the reaction conditions. This ensures that the equilibrium of the overall reaction will lie on the side of the desired esters or amides. Several different leaving groups have been introduced over the last decades, however, not all of them have been equally successful.



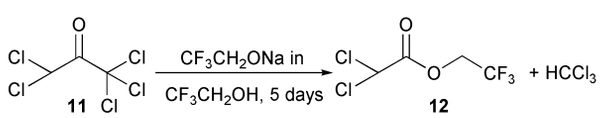
Scheme 8 Hydrolase-catalysed acylation of a primary amine or an alcohol utilising a quasi-irreversable acyl donor.

The 2,2,2-trichloroethyl alkanoates **7a** were used in the first enzymatic acylation studies in organic solvents.[5] The electron withdrawing effect of the chlorine atoms ensures that the released alcohol is a very weak nucleophile that cannot attack the product. Since 2,2,2-trichloroethyl groups are common protection groups for acids, numerous methods exist for the synthesis of **7a**.[37–39] It is actually surprising that a protecting group acts as an activating group. Indeed, the activation is very limited[3] and reactions are therefore slow. In the recent literature only very few examples of its application can be found, such as the regioselective formation of 6-*O*-dodecanoyl-D-glucono-1,5-lactone **10**, performed in neat pyridine (Scheme 9).[40]



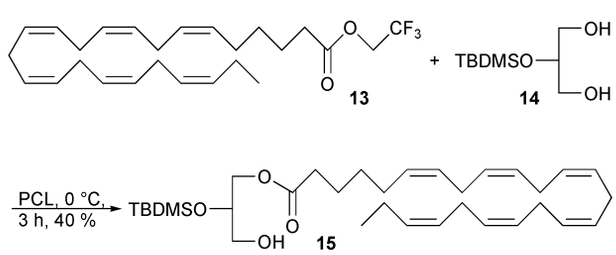
Scheme 9 *Porcine pancreas* lipase (PPL) catalysed regioselective acylation utilising a 2,2,2-trichloroethyl alkanoate.

2,2,2-Trifluoroethyl alkanoates **7b** are significantly more versatile than their trichloro equivalents **7a**. They are widely used and are readily prepared from the acids and the alcohol *via* carbodiimide coupling[41,42] or from the acid chlorides and the alcohol.[43,44] Recently **12** has been prepared *via* a haloform type reaction (Scheme 10); it has not yet been applied in an acylation reaction.[45] As an ester of a reactive acid (two chlorine atoms in the α-position) and an activating alcohol, **12** should open new opportunities for hydrolase-catalysed acylations.



Scheme 10 Synthesis of a potential acyl donor.

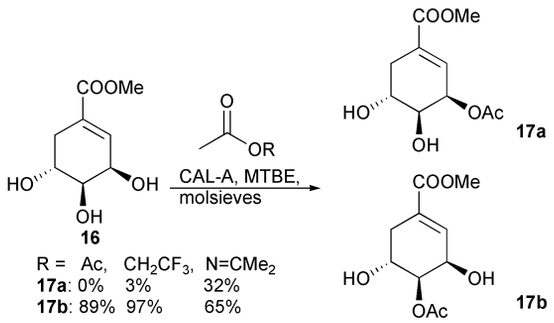
In a recent phosphatidylcholine synthesis the crucial coupling of a tetracosahexaenoic acid was achieved after 2,2,2-trifluoroethyl activation (Scheme 11),[42] and 2,2,2-trifluoroethyl butanoate was utilized for the *Candida antarctica* lipase A (CAL-A) catalysed kinetic resolution of β-aminoesters.[43]



Scheme 11 *Pseudomonas cepacia* lipase (PCL) catalysed acylation of a protected glycerol.

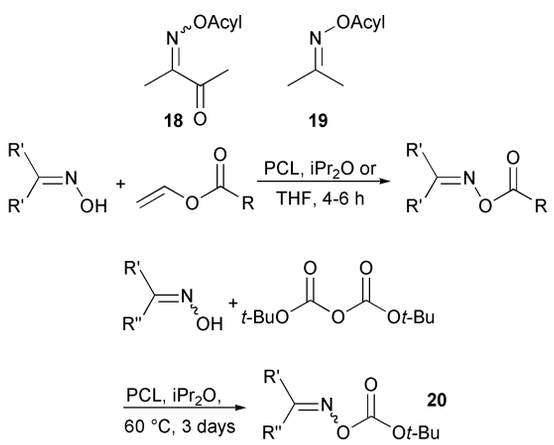
When comparing the reactivity of **7b** (R = Me) with that of **7c** (R = Me) and acetic anhydride in the regioselective acylation of shikimic acid methyl ester **16**, **7b** (R = Me) was as reactive as acetic anhydride and of equal selectivity whereas **7c** was significantly less reactive and reduced the selectivity of CAL-A (Scheme 12).[46]

The use of oxime esters **7c** as acyl donors in enzyme-catalysed reactions was first reported in 1989.[47] They are generally perceived to be irreversible acyl donors, although cases of reversible behaviour have been reported.[30] In general,



Scheme 12 Comparison of different acylating reagents in the CAL-A-catalysed regioselective protection of **16**.

two different types of oxime esters, **18** and **19**, are utilised. They can be prepared from the corresponding oximes and acid chlorides,[47] *via* DCC coupling of oximes and acids[48] and also *via* hydrolase-catalysed acylations of oximes.[49,50] ‡ Utilising lipases acyl donor **20** could be synthesized, which could then be applied in uncatalysed protection reactions of amino groups (Scheme 13).[50] Oxime acrylates of type **19** have been used to

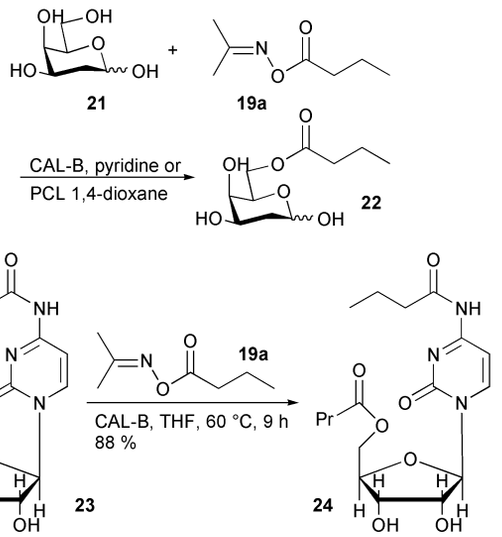


Scheme 13 Enzyme-catalysed synthesis of oxime esters.

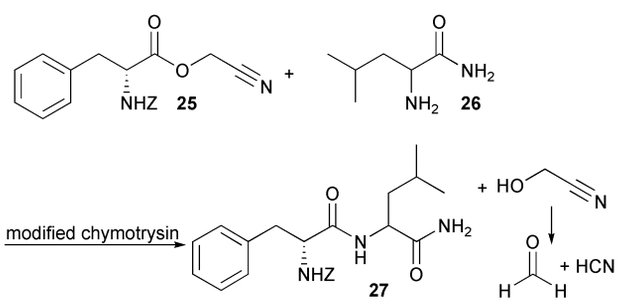
synthesize chiral acrylic acid esters. These esters formed the basis for chiral polymers[51] and, more recently, were the starting material for Diels–Alder reactions.[52] In a comparative study, type **18** oxime acrylate was more reactive than vinyl acrylate.[53] Oxime esters, in general, have successfully been used for the regioselective introduction of protecting groups on sugars[54] and nucleosides[55] (Scheme 14) and are popular acylating reagents[56,57] although the remaining oxime cannot always be removed easily.

The activation of acids in form of their cyanomethylesters **7d** has long been exploited in the synthesis of dipeptides.[58] These esters were, therefore, an obvious choice as reagents for hydrolase-catalysed acylations. They can be prepared readily from the acids and chloroacetonitrile in the presence of triethylamine.[58] Studies with chemically modified chymotrypsins showed that they could catalyse a series of amide couplings.[59,60] The cyanomethylester of Z-protected phenylalanine **25** was successfully coupled with both D and L leucine amide **26**; as expected the yields were lower for the non-natural D-**26** (Scheme 15). Although the reaction equilibrium was shifted to the product side, not many other applications of **7d** were reported,[61] which might be due to the toxicity of the HCN formed in the reaction mixture. At the same time the release of HCN also forms a fundamental difference between acyl donors **7a**–**c** and **7d**. The decomposition of the leaving groups ensures that the reaction can be turned into a truly irreversible acylation.

‡ This latter method might be one of the reasons why **7c** is not considered to be an irreversible acyl donor.



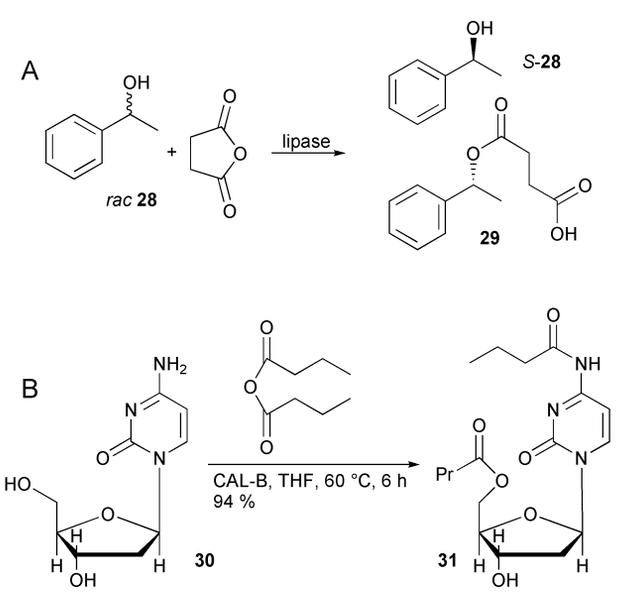
Scheme 14 Oxime esters as acylating reagents.



Scheme 15 Cyanomethylesters as acylating reagents.

3.3 Irreversible acyl donors

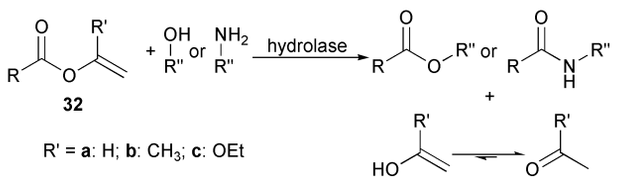
Just as in any chemical acylation, anhydrides can be used in enzyme-catalysed esterifications, albeit with a few drawbacks: the anhydrides can acylate the enzyme, deactivating it, the acid concentration in the reaction mixture is difficult to control, with might lead to undesired side reactions or to deactivation of the hydrolase and it is possible that an unselective background reaction occurs. Due to this, anhydrides have only found limited application in enzyme-mediated synthesis. Nonetheless succinic anhydride is used for the kinetic resolutions of alcohols,[62] even on an industrial scale (Scheme 16A).[33] Since **29** is an acid it can



Scheme 16 A: Industrial kinetic resolution of secondary alcohols; B: regioselective protection of deoxycytidine **30**.

readily be separated from *S*-**28**. Butyric anhydride was successfully utilised for the regioselective protection of deoxycytidine **30** (Scheme 16B).[55]

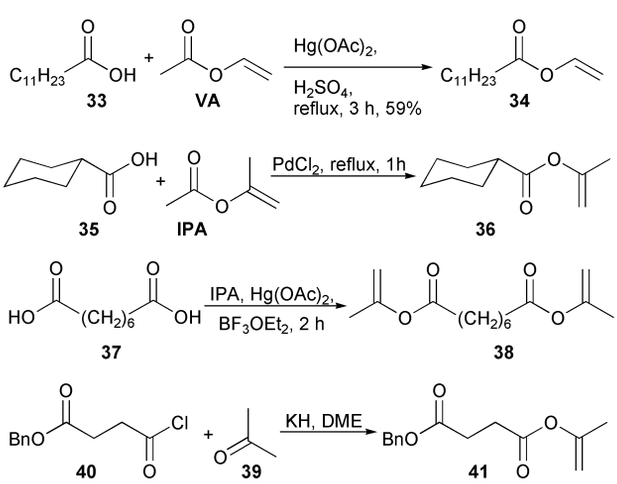
By far the most popular type of acyl donors are the enol esters **32a**–**c**. The leaving group is an enol that immediately tautomerises to the ketoform. Thus, no nucleophile remains and the reaction is irreversible (Scheme 17).



Scheme 17 Enol esters **32a**–**c** are irreversible acyl donors in hydrolase-catalysed C–O and C–N bond formations.

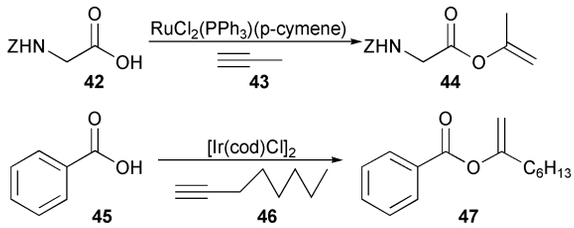
The application of vinyl esters **32a** and isopropenyl esters **32b** as acylating reagents in hydrolase-catalysed reactions was first described in 1987, both, in a patent[63] and in a paper.[64] Already in 1986 it had been briefly mentioned that **32b** could be used for enzymatic acylations,[65] the authors, however, only published a full account on their method in 1988.[66]

Many vinyl esters **32a** and isopropenyl acetate (IPA) are commercially available, since they are building blocks in polymer chemistry or are used as acylating reagents in non-enzyme-catalysed reactions.[67] These bulk chemicals can readily be converted into the desired vinyl[68] or isopropenyl esters by (Lewis) acid[69,70] or palladium-catalysed[71,72] transesterifications. Alternatively, the potassium enolate of acetone in DME can be treated with the acid chlorides, yielding **32b** in moderate to good yields (Scheme 18).[73]



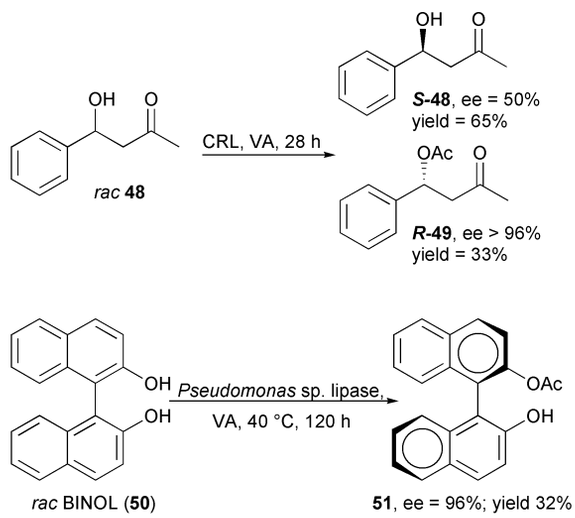
Scheme 18 Synthesis of vinyl and isopropenyl esters **32a**,**b**.

The synthesis of enol esters, starting from the acids and acetylenes, can be achieved with different ruthenium catalysts.[74,75] These catalysts show high Markovnikov selectivity[76] and have been immobilised.[77] Recently it was shown that $[Ir(cod)Cl]_2$ catalyses the formation of vinyl and isopropenyl esters, too (Scheme 19).[78] The Ru- and Ir-catalysed syntheses of enol esters proceed under gentle conditions, the yields, however, vary greatly and are similar to those of the transesterifications.



Scheme 19 Ru[76] and Ir[78] catalysed synthesis of enol esters.

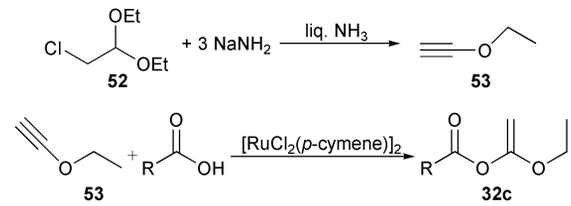
Many of the enzyme-catalysed kinetic resolutions are acylations of a multitude of substrates utilizing either vinyl acetate (VA) or IPA. Just two recent examples are given to highlight the vast number of possibilities that these acyl donors provide to organic chemists. When VA is used as a solvent CRL selectively acylates only one enantiomer (***R*-48**) of β-hydroxyketone *rac* **48**.[79] This straightforward preparation of an enantiopure aldol product *via* an unselective aldol reaction and a kinetic resolution can easily compete with the proline-catalysed synthesis of the same product.[80] Optically pure BINOL **50**, the backbone of many chiral transition metal catalysts, is normally obtained *via* resolution. *Pseudomonas* sp. lipase can be used to acylate **50**, yielding virtually enantiopure **51** (Scheme 20).[81]



Scheme 20 Recent successful applications of enol esters in lipase-catalysed kinetic resolutions.

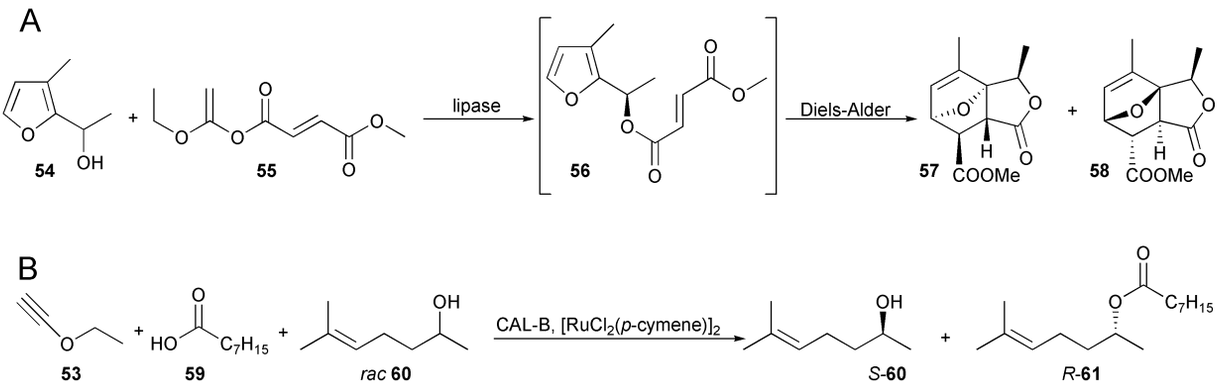
Initially it was thought that the acetaldehyde released during the reactions with VA could be detrimental to the hydrolases. Indeed, it can form imines with lysine residues on the surface of the enzymes.[82] Fortunately, only two of the commonly used lipases are somewhat sensitive to this problem, CRL and *Geotrichium candidum* lipase (GCL). Even though this sensitivity was observed, CRL can be used in VA as solvent.[79] Moreover, CRL can be stabilised by immobilisation on epoxy resins.[83,84]

Ethoxyvinyl esters as acyl donors in enzyme catalysed reactions **32c** were first described in 1996.[85,86] They were shown to be as reactive as VA (*i.e.* more reactive than IPA), while having the advantage that the leaving group, ethyl acetate, is not harmful.[87] Ethoxyvinyl esters **32c** can readily be prepared by addition of an acid to ethoxyacetylene **53**. The reaction is catalysed by the commercially available Bennett's ruthenium complex.[88] Although **53** is commercially available, it is expensive and better results are obtained with samples freshly prepared from chloroacetaldehyde diethyl acetal **52** (Scheme 21).[89,90]



Scheme 21 Preparation of ethoxyvinyl esters **32c**.

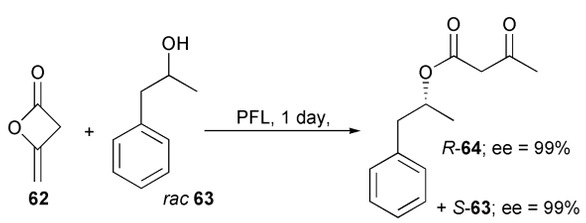
The great ease of their preparation under mild conditions makes ethoxyvinyl esters **32c** an ideal tool for the introduction of complex acid moieties into the target molecules of the enzyme-catalysed reaction.[91,92] In the case of activated fumaric acid even a Diels–Alder reaction of the generated ester pro-



Scheme 22 A: Lipase-catalysed acylation and Diels–Alder reaction; B: one-pot activation and esterification of an acid.

ceeded in the reaction mixture, modified by the same lipase that catalysed the ester synthesis (Scheme 22A).[93] Indeed, the Ru-catalysed activation of acids occurs under such mild conditions that it can be performed *in situ* in the presence of the enzyme. This one-pot procedure for activation and acylation is a significant step forward (Scheme 22B).[94] Hydrolase-catalysed esterifications and amidations can now be achieved directly from the acid and the alcohol or amine, similar to a DCC coupling. Moreover, the amount of waste generated is much smaller than in non-enzyme-catalysed reactions. In addition to the higher atom efficiency, this also eases the work up considerably.

Similar to enol esters, enol lactones can be utilized for the irreversible acylation of alcohols and amines. In 1993 diketene **62** was described as a versatile reagent for the enantioselective synthesis of acetoacetates.[95,96] Although very good enantioselectivities were described for *Pseudomonas fluorescens* lipase (PFL) catalysed kinetic resolutions of alcohols (Scheme 23),[97] diketene **62** has not become a generally applied acylating reagent. This might be due to its highly reactive nature, or to the fact that acetoacetates can be prepared with great ease from methyl or ethyl acetoacetate.[98]



Scheme 23 Diketene **62** as an irreversible acylation reagent.

3.4 Activated carbonates as acyl donors

Carbonates, carbamates and urethanes are common structural elements in natural products and drugs. Moreover, they are versatile protection groups that often need to be introduced regioselectively.[29] During the last decade several different activated carbonates have been developed for the enzyme-catalysed formation of these functional groups.[99]

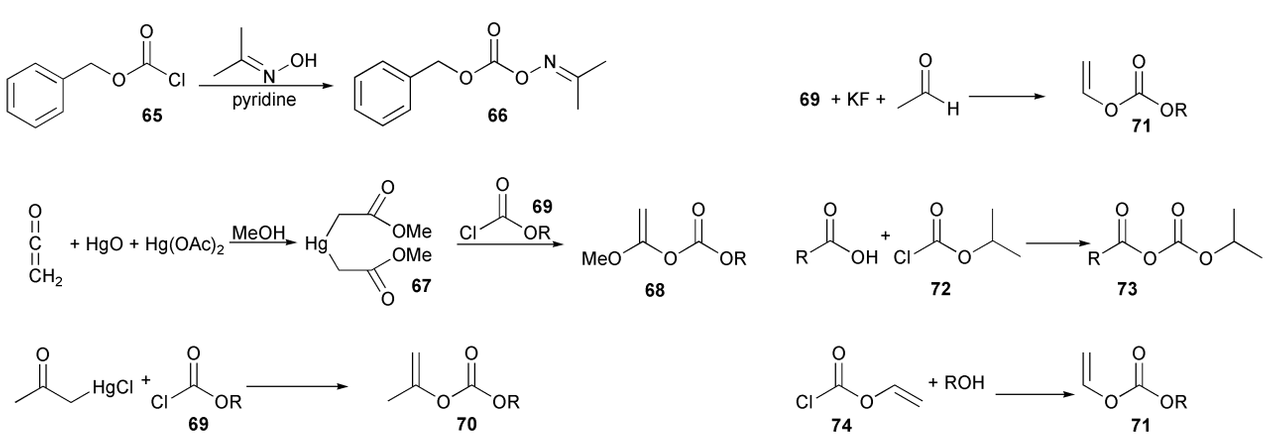
Activated carbonates can be used for the introduction of these important building blocks. The activated carbonates can be synthesised by the addition of acetone oxime,[100] ketene,[101] acetone enolate equivalents,[102] acetaldehyde enolate[103] or acids[104] to chloroformates to yield oxime esters **66**, ethoxyvinyl esters **68**, isopropenyl esters **70**, vinyl carbonates **71** or mixed anhydrides **73**, respectively. Alternatively, vinyl carbonates **71** can be synthesised from vinyl chloroformate **74** and an alcohol[105–108] (Scheme 24). The di-activated vinyl oxime carbonate **76** can be generated from **74** and acetone oxime in almost quantitative yield.[109] A lipase-catalysed formation of **20** was already described earlier (Scheme 13).[50]

When vinyl carbonates **71** are applied in lipase-catalysed acylation of alcohols[105,107] or amines,[106–108] the corresponding carbonates or carbamates are obtained in very good yields and selectivities under mild reaction conditions. In the case of the mixed anhydrides **73** the main product are the esters of the acid and not the carbonates.[104]

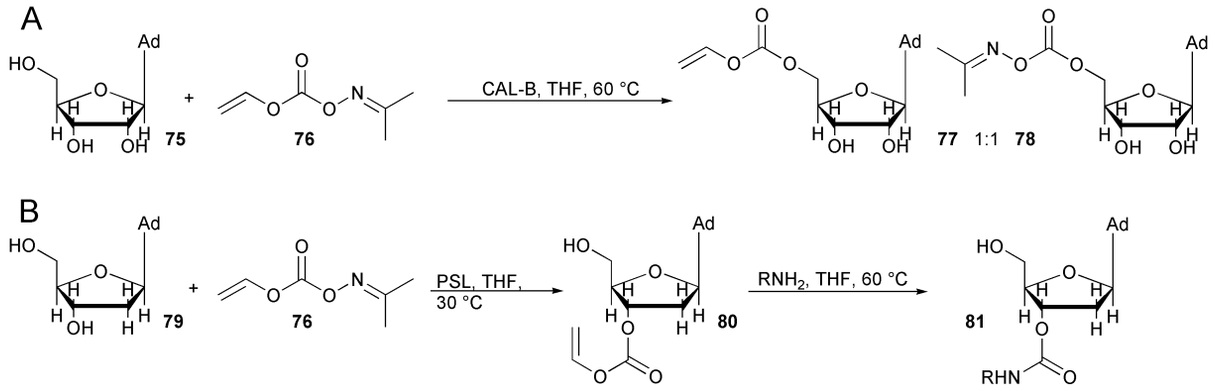
When the di-activated carbonate **76** is utilized, two reactions can proceed in a cascade whilst at the same time it is possible to determine which of the two activating groups is more reactive. Using **76** it could be demonstrated that they activated the carbonate equally well (Scheme 25A).[109] Under modified reaction conditions, the oxime ester was, however, significantly more reactive and the vinyl ester was converted in a second, non-catalytic step, into a carbamate (Scheme 25B).[110] This indicates the importance of the exact control of the reaction conditions. **76** has also been successfully utilized in the synthesis of vitamin D precursors.[111,112]

3.5 How irreversible are irreversible acyl donors?

Ideally, one equivalent of acyl donor should react with one equivalent of alcohol or amine to yield 100% ester or amide. Is

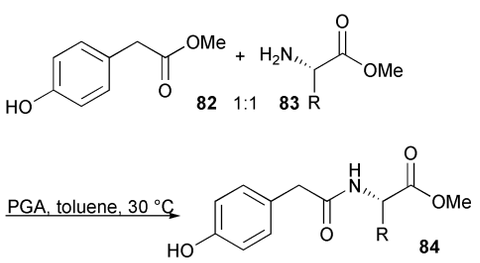


Scheme 24 Syntheses of activated carbonates.



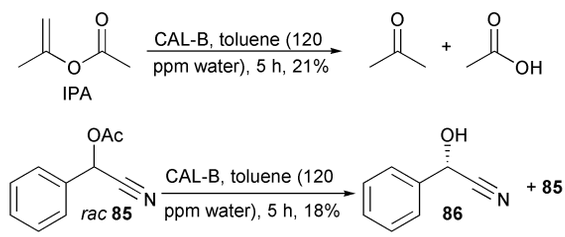
Scheme 25 Applications of activated carbonates in synthesis.

this indeed the case? A considerable difference between the formation of esters and amides can be noticed. In general the synthesis of amides proceeds with ease. This is partly due to the fact that amines are significantly better nucleophiles than alcohols. Another reason might be that the proteases and amidases often utilised in these reactions seem to be less prone to using water as a nucleophile, thereby ensuring that the equilibrium is entirely on the product side. Reversible and quasi-irreversible acyl donors, such as **1** and **7a**–**d**, give good to excellent yields. Even normal esters can be used as acyl donors. Indeed, the penicillin G acylase (PGA) catalysed synthesis of several commercial β-lactam antibiotics is performed with esters as acyl donors in aqueous media. The synthesis (*i.e.* attachment of the penicillin side chain) to hydrolysis (*i.e.* hydrolysis of the ester) ratios for these processes have been carefully determined and optimised towards the synthesis reaction.[113] When PGA is utilised in toluene with a low water concentration the formation of amides **84** can be achieved with 98% yield,[114] for both, the acyl donor and the amine (Scheme 26). Recently an efficient, enzymatic synthesis of a pentapeptide was reported. The formation of all the amide bonds was catalysed by chymotrypsin, papain or thermolysin, demonstrating the great potential of hydrolases for these reactions.[115]



Scheme 26 Highly efficient amide synthesis.

Does the hydrolase-catalysed acylation of alcohols proceed with an efficiency similar to that of the amide formation? In 1995 doubt was cast on the irreversibility of VA as an acyl donor. It was shown, that the enzyme (PFL) also catalysed the deacylation in the same reaction. Small quantities of water, always present in the reaction mixture, were one of the causes. It was recommended to work as dry as possible and to monitor the reaction carefully, since most of the deacylation took place towards the end of the reaction.[116] When the CAL-B-catalysed acylation of 1-phenylethanol **28** with VA was monitored by NMR, it became obvious that the concentration of VA decreased considerably faster than the concentration of **28**. A significant part of VA was hydrolysed by the traces of water in the dry solvent.[117] This implies that acetic acid is formed in the reaction mixture. It has been reported that the addition of non-nucleophilic bases improves the selectivity of lipases.[118,119] This might be due to the fact that they neutralize the released acid, preventing changes in the ionisation state of the enzyme. In an investigation of a dynamic kinetic resolution it was recently demonstrated that even in dry toluene (120 or 60 ppm water) both, the acyl donor (IPA) and the product **85** were hydrolysed by CAL-B (Scheme 27).[120] Since the amount of



Scheme 27 CAL-B-catalysed hydrolysis in dry solvents.

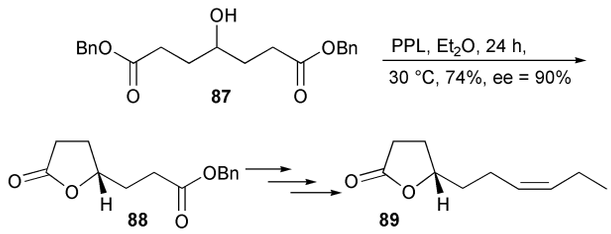
water in the solvent did not allow for the extent of hydrolysis, this does imply that water introduced with the enzyme and its carrier participated in the reaction. Based on these findings it has to be concluded that for the formation of esters an excess of acyl donor should be used. Although this could have detrimental effects on the yield (when calculated for the acyl donor) with the *in situ* synthesis and application of ethoxyvinyl esters as described in Scheme 22B this problem can be avoided.

4 Acyl donors in de-racemisation and de-symmetrisation reactions

One of the main applications of acyl donors is in the kinetic resolutions of alcohols, amines and acids. Kinetic resolutions of racemates can, by definition, yield no more than 50% of the desired product. Moreover, they are only necessary when the planned reaction in the synthesis was entirely unselective, yielding the undesired racemate. From a synthetic point of view, kinetic resolutions are the additional one or two steps that are needed in order to obtain enantiopure product from a non-selective reaction (with a theoretical yield of 50%).

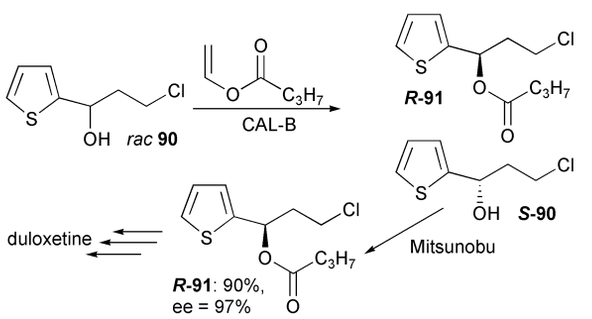
The development of techniques that allow for yields of up to 100% from kinetic resolutions has, therefore, attracted great interest. If possible, the acylation should be performed in such a way (possibly coupled with other transformations in the same flask) that the formation of the acylated product is also the desired synthetic step. Several strategies to achieve this goal exist.[121] The *meso*-trick is a straightforward approach to achieve this target. A *meso* compound is submitted to a regioselective acylation and the product obtained (up to 100%) is chiral. One of the two equivalent groups (acid, alcohol or amine) is protected or converted into the desired chiral ester/amide. The same strategy can be applied to symmetrical starting materials such as **87**, *e.g.* it was successfully utilized in the synthesis of (−)-γ-jasmolactone **89** (Scheme 28).[122]

A more general approach is the coupling of a kinetic resolution with a Mitsunobu inversion.[123] After acylating one enantiomer the remaining, unreacted enantiomer is inverted,



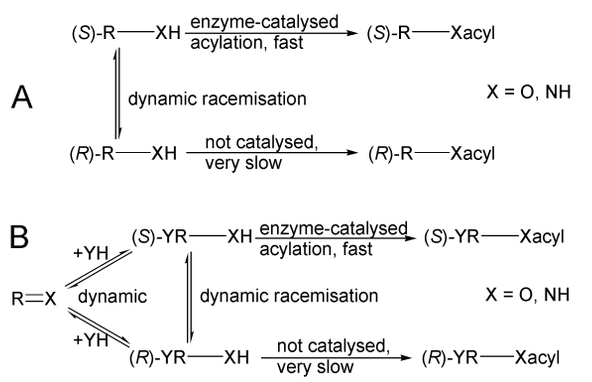
Scheme 28 Synthesis of (−)-γ-jasmolactone from a symmetrical starting material.

yielding 100% of the enantiopure product. This enantio-convergent process has recently been applied in the synthesis of duloxetine (Scheme 29).[124]



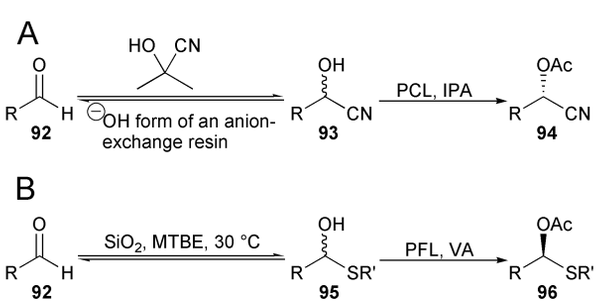
Scheme 29 Enantio-convergent approach towards duloxetine.

Of equal, or even larger, synthetic utility are dynamic kinetic resolutions. This is the coupling of two reactions, a kinetic resolution and a dynamic equilibrium between the two enantiomers of the starting material, *i.e.* a racemisation. If the racemic starting material of a kinetic resolution is kept racemic throughout the reaction (*via* a rapid racemisation, possibly *via* a prochiral intermediate) then 100% yield can be achieved in this type of resolution (Scheme 30).



Scheme 30 Dynamic kinetic resolutions.

The first dynamic kinetic resolution that was based on an enantioselective acylation is a show case example of the great synthetic power of this approach (Scheme 31A). Instead of

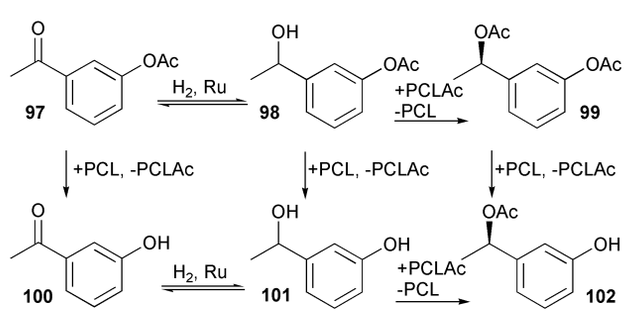


Scheme 31 Dynamic kinetic resolutions that are equivalent to one enantioselective synthetic step.

utilising a racemic compound, the prochiral "intermediate" was used as the starting material (Scheme 30B).[125,126,120] Aldehyde **92** is converted into the racemic cyanohydrin **93**, *via* a dynamic, base-catalysed equilibrium. The PCL-catalysed, enantio-selective acylation forms the overall product, *S*-**94**, in excellent yields and optical purities (Scheme 31A). It has been reported that CRL enantioselectively catalyses the formation of *R*-**94**, *i.e.* in principle both enantiomers can be obtained.[127,128] Only very recently a transition-metal-catalysed equivalent to this synthesis with concurrent enantioselective protection of unstable cyanohydrins was reported.[129] Another example of the synthetic versatility of this approach is the enantioselective formation of hemithioacetal acetates **96** directly from aldehydes **92** (Scheme 31B).[130] Both reactions (31A/B) proceed according to Scheme 30B and utilize the prochiral "intermediate" as starting material. The reactions are, therefore, not just kinetic resolutions with 100% yield but equivalent to stereoselective synthetic reactions during which new C–C or C–S bonds are formed. This is in contrast to dynamic kinetic resolutions that proceed *via* Scheme 30A or Scheme 30B with the racemic compound as the starting material.

The dynamic kinetic resolution of secondary alcohols has attracted considerable attention. In their landmark paper Williams *et al*,[131] demonstrated that iridium, rhodium, ruthenium and aluminium triisopropoxide could be utilised for the racemisation of secondary alcohols and established the first dynamic kinetic resolution for this class of compounds. This system works according to Scheme 30A. It is a kinetic resolution with 100% yield, but it is not equivalent to a bond-forming step. Great improvements were achieved by modifying the ruthenium catalyst (racemisation) and by introducing *p*-chlorophenol acetate§ as an acyl donor.[132] *p*-Chlorophenol acetate is not necessarily a particularly good acyl donor, but it does not interfere with the ruthenium catalysts. A recent review by Bäckvall *et al.* summarizes the major contributions of his group and others active in this field.[133] The first examples of this system still needed large amounts of ketone (acetone or the ketone that corresponds to the secondary alcohol) to be added to the reaction mixture; this can, however, be avoided by performing the reaction in the presence of oxygen.[134] Recently, it was demonstrated that even the dynamic kinetic resolution of alcohols can be performed as equivalent to the enantioselective reduction of a ketone and its *in situ* protection. **97** was treated under a hydrogen atmosphere with a ruthenium catalyst. This established the dynamic equilibrium between ketone **97** and alcohol **98** and also between **100** and **101**. PCL-catalysed the deacylation of **97**, **98** and **99**. Subsequently PCL transferred the acyl group enantioselectively onto the newly formed alcohol groups in **98** and **101**. **102** was obtained in excellent yield and enantiopurity (Scheme 32).[135]

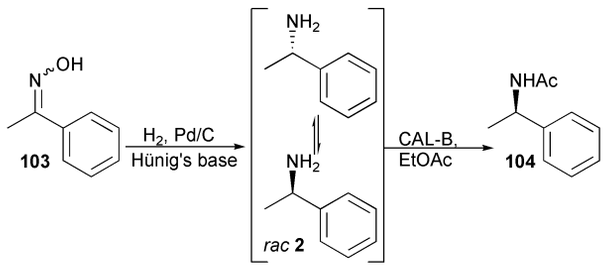
Similar advances have been made in the dynamic kinetic resolutions of amines. While the first example followed Scheme



Scheme 32 Enantioselective formation of a secondary alcohol acetate.

§ *p*-Chlorophenol acetate can readily be prepared from *p*-chlorophenol and acetic anhydride. Due to its unpleasant and persistent smell *p*-chlorophenol is not utilised in the teaching laboratories here in Delft any longer.

30A,[136] a recent example utilizes oxime **103** as prochiral starting material, *i.e.* follows Scheme 30B. Again, it was possible to achieve a synthetic reaction step (equivalent to an enantioselective reduction of an oxime to an amine), rather than a kinetic resolution with 100% yield (Scheme 33).[137]



Scheme 33 Enantioselective formation of a primary amine acetate. This dynamic kinetic resolution is equivalent to an enantioselective synthetic step (enantioselective reduction of an oxime to an amine).

5 Conclusions and perspectives

During the last two decades the methodology for irreversible hydrolase-catalysed acylations of alcohols and amines has been developed. Many different activated acid derivatives have been introduced, giving chemists a wide choice of starting materials. This, together with the commercially available enzymes, hands the chemist tools that are necessary for his trade. Especially the synthesis of amides and peptides can now readily be achieved by regioselective enzymatic coupling reactions. A possible drawback in the formation of esters is the lipase-catalysed hydrolysis of both the acyl donor and the ester that proceeds as a background reaction. This problem can be circumvented by utilizing an excess of the acyl donor (if readily/commercially available) or by applying the coupled ruthenium-catalysed activation and *in situ* acylation methodology (Scheme 22B). With the development of (doubly) activated carbonates, the synthetically important carbonates, carbamates and urethanes can now readily be introduced into the target molecule, while at the same time utilising the enantioselectivity of the enzymes to set up the desired stereochemistry.

The great regioselectivity of the hydrolases enables the directed introduction of protection groups onto sugars and peptides; their enantioselectivity has in the first instance been utilized in kinetic resolutions. With the introduction of the *meso*-trick, the combination of kinetic resolutions with Mitsunobu inversions (Scheme 29) and synthetic dynamic kinetic resolutions (Scheme 30B, R=X as starting material), irreversible acylations are now equivalent or even superior to other, non-enzyme-catalysed, enantioselective synthetic procedures. At the same time this is a field where many further developments can be expected. Given the vast number of reversible reactions during which amino- and hydroxy-groups are generated—such as the aldol reaction¶—the possibilities for new dynamic kinetic resolutions as tools for synthesis seem almost unlimited.

With the investigation of the biosynthesis of polyketides and nonribosomal peptides a large number of thioesterases has become available. These are nature's catalysts for macrolactonisation.[138,139] While the lipase-catalysed synthesis of lactones has only shown limited success, in part due to the formation of dimers and trimers,[140] the thioesterases might help to solve this problem, thereby shifting from being a research topic in biochemistry to one in pure chemistry.

Looking back at Emil Fischer's assessment of the potential of enzymes in chemistry from 1894 only one conclusion is possible: He was right. Enzymes, and especially hydrolases, are versatile tools for organic synthesis. They are readily available and straightforward to use. It is up to today's chemists to decide whether enzymes are part of biology or pure chemistry, as Emil Fischer stated.

¶ Attempts to establish a dynamic equilibrium between acetone and benzaldehyde or isobutyraldehyde with solid buffers[10,11] have so far not been successful; M. Kastrin, D. Kikelj and U. Hanefeld, unpublished results.

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