# Enzymatic Protecting Group Techniques

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## *Contents*



## *I. Introduction*

The proper introduction and removal of protecting groups is one of the most important and widely carried out synthetic transformations in preparative organic chemistry. In particular, in the highly selective construction of complex, polyfunctional molecules, e.g., oligonucleotides, oligosaccharides, peptides, and conjugates thereof, and in the synthesis of alkaloids, macrolides, polyether antibiotics, prostaglandins, and further natural products, regularly the problem arises that a given functional group has to be protected or deprotected selectively under the mildest conditions and in the presence of functionalities of similar reactivity as well as in the presence of structures being sensitive to acids, bases, oxidation, and reduction. For the manipulation of protecting groups, numerous classical chemical methods have been developed.[1−3] Nevertheless, severe problems still remain caused by the need to selectively introduce or remove specific blocking functions which cannot or can only with great difficulties be solved by using classical chemical tools only. However, the arsenal of available protecting group techniques has been substantially enriched by the application of biocatalysts. In addition to their stereodiscriminating properties, enzymes offer the opportunity to carry out highly chemo- and regioselective transformations. They often operate at neutral, weakly acidic, or weakly basic pH values and in many cases combine a high selectivity for the reactions they catalyze and the structures they recognize with a broad substrate tolerance. Therefore, the application of these biocatalysts to effect the introduction and/or removal of suitable protecting groups offers viable alternatives to classical chemical methods.[4−11]

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## *II. Protection of Amino Groups[4−12]*

### A. N-Terminal Protection of Peptides

The selective protection and liberation of the α-amino function, the carboxy group, and the various side chain functionalities of polyfunctional amino acids constitute some of the most fundamental problems in peptide chemistry. Consequently, numerous efficient protective functions based on chemical techniques were developed to a high level of practicability.[1−3,13,14] However, since the mid-1970s, a systematic search for blocking groups that are removable with a biocatalyst has been carried out.[4−12] In addition to the mild deprotection conditions they promise, protecting groups of this type are expected to be particularly useful for the construction and manipulation of larger peptide units, i.e., for transformations which, for solubility reasons, in general have to be carried out in aqueous systems. Also, applications in the reprocessing of peptides obtained by recombinant DNA technology are foreseen.

Initial attempts to introduce an enzyme-labile amino protecting group involved the use of chymotrypsin for the removal of *N*-benzoylphenylalanine (Bz-Phe) from the tripeptide Bz-Phe-Leu-Leu-OH.[15]

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Dieter Kadereit, born in 1971 in Bremen (Germany), began studying chemistry in 1991 in Hannover. After investigating (1994/95) the rhodium-(I)-catalyzed cycloaddition in the group of P. A. Wender at Stanford University, he received (1996) his diploma in Hannover and Aachen, studying manganese-catalyzed asymmetric oxidation reactions. In 1997 he joined the group of H. Waldmann to work on the synthesis and evaluation of lipidated peptides. After graduation in 2000 he joined Aventis AG, Frankfurt.

Herbert Waldmann, born in 1957, received his Dr. rer. nat. degree in 1985 (Universität Mainz, H. Kunz). After postdoctoral studies (1985–1986, Harvard University, George Whitesides) and habilitation (1991, Universität Mainz), he accepted a professorship at the Universität Bonn in 1991. In 1993 he moved to the Universität Karlsruhe as Full Professor of Organic Chemistry. In 1999 he was appointed as Director at the Max-Planck-Institut of Molecular Physiology, Dortmund (Department of Chemical Biology), and Full Professor of Biochemistry at the University of Dortmund. Herbert Waldmann has been the recipient of the Friedrich Weygand Award for the advancement of peptide chemistry, the Carl Duisberg Award of the Gesellschaft Deutscher Chemiker, the Steinhofer Award of the Steinhofer Foundation, and the Otto Bayer Price. His current research interests include bioorganic chemistry and natural product synthesis as well as biocatalysis, stereoselective synthesis, and combinatorial chemistry. A major focus of his research activities is on the combination of organic chemistry, biophysics, and biology for the synthesis and biological evaluation of peptide and protein conjugates which are involved in biological signal transduction processes. Most recently, syntheses of natural products and natural product-derived compound libraries on polymeric supports have been investigated by the Waldmann group.

The desired dipeptide H-Leu-Leu-OH was obtained in 80% yield under mild conditions (pH 7.3, room temperature). Chymotrypsin, however, is an endopeptidase of rather broad substrate tolerance, catalyzing the hydrolysis of peptide bonds on the carboxy groups of hydrophobic and aromatic amino acid residues. Since such amino acids appear widely in peptides and since no method is available to protect them against attack by the enzyme during the attempted deprotection, the use of chymotrypsin

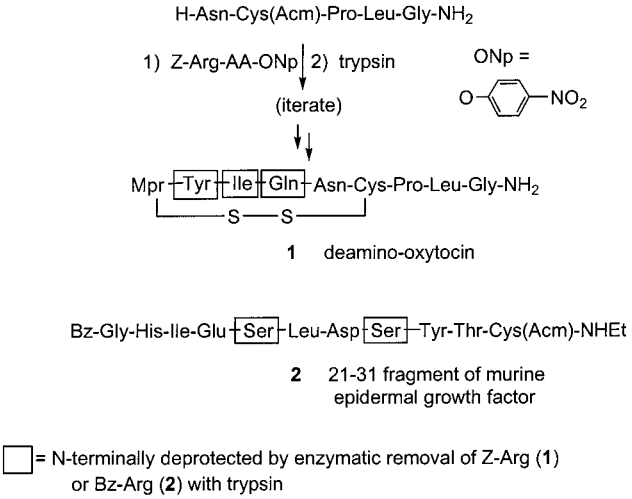



**Figure 1.** Construction of oligopeptides via removal of N-terminal arginine residues with trypsin.

is problematic. It will, therefore, be limited to special cases[16] in which no danger of competitive cleavage at undesired sites has to be feared. A protease of much narrower specificity is trypsin, which catalyzes the hydrolysis of peptide bonds at the carboxylic group of lysine and arginine. These amino acids carry polar, chemically reactive side chain functional groups which can be protected by various techniques.[13,14] The sharp specificity of trypsin together with the possibility of hiding the critical amino acids which function as primary points of tryptic cleavage allowed for the development of a broadly applicable system for the protection of the α-amino group of peptides.[12,17−19] In several studies the application of trypsin-labile protecting groups along with suitable blocking functions for the side chains of arginine and lysine were described.[17−23] Thus, for instance, Z-Arg-OH served as an enzymatically removable protecting group in a stepwise synthesis of deamino-oxytocin **1** (Figure 1).[18,19]

Starting with a pentapeptide, the amino acid chain was elongated with Z-Arg-protected amino acid *p*-nitrophenyl esters. The N-terminal Z-Arg protecting group was successively removed in moderate to high yield and without attack on the other peptide bonds by treatment with trypsin. Unfortunately, preparation of the protected arginine *p*-nitrophenyl esters is difficult, thus preventing this method from becoming generally useful for the stepwise assembly of larger peptides. The trypsin-labile blocking groups have, however, proven to be very useful for the construction of oligo- and polypeptides via condensation of preformed peptide fragments. An illustrative example consists of a chemoenzymatic construction of the 21−31 fragment **2** of murine epidermal growth factor (Figure 1). In the course of this synthesis the deblocking by trypsin was applied twice.[16] The enzyme first liberated the N-terminus of a tetrapeptide and subsequently of a heptapeptide. In the synthesis[24] of human β-lipotropin, an Ac−Arg residue was introduced by a solid-phase technique at the N-terminus of the 29 C-terminal amino acids of the desired polypeptide. After cleavage from the resin and protection of the side chain functionalities, the arginine moiety was removed with trypsin, leaving the peptide

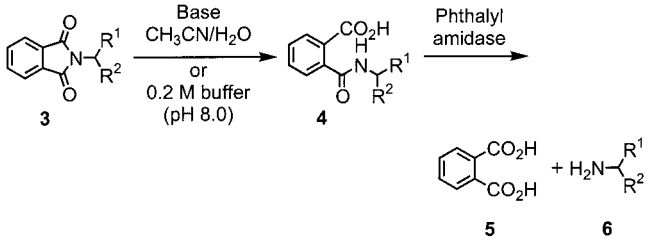



**Figure 2.** Enzymatic removal of the phthalyl group.

chain intact. Finally, coupling of this 61−89 fragment to a partially protected 1−60 segment and subsequent deprotection delivered $\beta$-lipotropin. Further examples are found in syntheses of oxypressin,[12] Met-enkephalin,[25] and Glu$^4$-oxytocin.[12]

In addition to chymotrypsin and trypsin, the collagenase from *Clostridium histolyticum* has been proposed as a catalyst for the removal of N-terminally attached dummy amino acids from peptides.[26] The enzyme recognizes the tetrapeptides Pro-X-Gly-Pro and splits the X-Gly bond. The use of this biocatalyst permitted the construction of des-pyroglutamyl-[15-leucine]human little gastrin I by selective hydrolysis of the dipeptide Pz-Pro-Leu (Pz = 4-phenylazobenzyloxycarbonyl) from the N-terminus of the octadecapeptide Pz-Pro-Leu-Gly-Pro-Trp-Leu-(Glu)$_5$-Ala-Tyr-Gly-Trp-Leu-Asp-Phe-$NH_2$. Transformations of this type resemble an analogy to the naturally occurring conversion of prohormones into hormones and may prove to be useful for the processing of peptide factors produced by recombinant DNA technology.

Despite the impressive syntheses that have been realized using proteases, the use of these enzymes is always accompanied by the danger of a competitive (and sometimes unexpected and unforeseeable) cleavage of the peptide backbone at an undesired site. At a minimum, complex protecting group schemes may become necessary if the amino acid which serves as recognition structure for the protease occurs several times in the peptide chain to be constructed. This disadvantage can be overcome if a biocatalyst devoid of peptidase activity is used for the liberation of the N-terminal amino group. This principle has been realized by applying penicillin G acylase from *E. coli*,[27−44] which is used in industry for the large-scale synthesis of semisynthetic penicillins and by using a phthalyl imidase from *Xanthobacter agilis*[45−47] (vide infra). Penicillin G acylase attacks phenylacetic acid (PhAc) amides and esters but does not hydrolyze peptide bonds. It accepts a broad range of protected peptides as substrates and selectively liberates the N-terminal amino group under almost neutral conditions (pH 7−8, room temperature), leaving the amide bonds as well as the C-terminal methyl, allyl, benzyl, and *tert*-butyl esters unaffected.[28−35,38] The PhAc group is easily introduced into amino acids by chemical[48] or enzymatic[49] methods and is stable during the removal of the C-terminal protecting groups employed.[29−32]

Recently, it has been shown that a phthalyl amidase isolated from *Xanthobacter agilis* is able to deprotect a variety of phthalimido substrates once the substrates are partially hydrolyzed to their monoacids (Figure 2).[45−47] The phthalyl group is

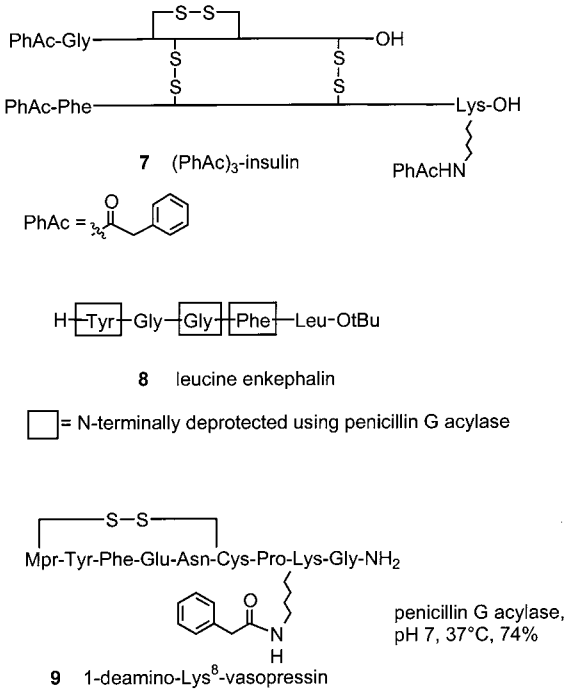



**Figure 3.** Application of the phenylacetamido (PhAc) group as an enzymatically removable amino protecting group.

commonly used for amine protection, because it completely blocks this functionality by double acylation.[2,3] The enzymatic phthalyl removal proceeds via a two-step process by weakly basic hydrolysis to yield the monoacid **4** and subsequent treatment with the phthalyl amidase (Figure 2). Especially because the hydrolysis of the phthalimide **3** to the corresponding monoacid **4** can be catalyzed by imidases such as the rat liver imidase,[50] this procedure represents a powerful alternative to the classical phthalyl deprotection which requires relatively drastic conditions and toxic reagents. However, the general applicability of the enzymatic phthalyl removal remains to be investigated.

If the construction of PhAc- or phthalyl-peptides is carried out by chemical activation of the PhAc-amino acids, the application of the non-urethane blocking group results in ca. 6% racemization.[29,30] However, this disadvantage can be overcome by forming the peptide bonds enzymatically, e.g., with trypsin,[51] chymotrypsin,[51] or carboxypeptidase Y,[39,51] or by using urethane-type protecting groups (vide infra). For such condensation reactions and the subsequent enzymatic removal of the PhAc group, a continuous process was developed which has the potential to be amenable to a larger scale.[39]

The applicability of the penicillin acylase-catalyzed deprotection for the construction of larger peptides has been demonstrated in the complete deprotection of the porcine insulin derivative **7** carrying three PhAc groups,[27] presumably at the N-terminal glycine of the A-chain, the N-terminal phenylalanine of the B-chain, and the side chain of the lysine in position 29 of the B-chain (Figure 3). The enzymatic hydrolysis proceeded to completeness, and the peptide backbone was not attacked. A further interesting example is given in a recent biocatalyzed synthesis of leucine

enkephalin *tert*-butyl ester **8**[38] in which all critical steps are performed by enzymes, two of them through the agency of penicillin G acylase: (i) phenylacetates are introduced as N-terminal protecting groups of the amino acid esters by using penicillin G acylase, (ii) the elongation of the peptide chain is carried out with papain or α-chymotrypsin, (iii) the deprotection of the N-terminal amino group is achieved again by means of penicillin G acylase. These examples and also the application of this technique for aspartame syntheses,[28,40,41] as well as the deprotection of glutathione derivatives,[35] demonstrate that penicillin G acylase can advantageously be used for the N-terminal unmasking of peptides. In addition, the enzyme has been used for the liberation of the side chain functionalities of lysine and cysteine as well as in *β*-lactam, nucleoside, and carbohydrate chemistry (vide infra).

## B. Enzyme-Labile Urethane Protecting Groups

The enzyme-labile N-protecting functions described so far are simple acyl groups which embody the danger of racemization during chemical peptide syntheses. This problem can, in general, be overcome by the use of urethane blocking functions. However, so far very few examples of a biocatalytic removal of classical urethane protecting groups such as the Z and Boc group are known.[52] Apparently the enzymatic attack on urethane carbonyl group, which would initiate the cleavage process, is also inefficient to be synthetically useful. To overcome this problem, two different strategies were developed. Both concepts have in common that the enzyme-labile bond is no longer part of the urethane. Instead, the first approach includes the introduction of a spacer (the AcOZ- and PhAcOZ groups), while the second strategy relies on the cleavage of a glycosidic C−O bond of a glycoside urethane by the respective biocatalyst, e.g., a glucosidase (the BGloc group).

By introduction of a spacer between the group which is recognized by the enzyme and the urethane, the substrate is kept at a distance from the enzyme during the reaction (Figure 4). Therefore, any steric effects caused by the bulk of certain amino acids are expected to be minimal and, as the amino acid sequence does not influence the reactivity, this concept should be generally applicable for the synthesis of peptides and peptide conjugates. An additional advantage of the spacer introduction is the option to choose the group which is recognized by the enzyme and thereby the enzyme itself.

This concept was first realized by using *p*-hydroxybenzyl alcohol as a spacer in the *p*-(acetoxy)benzyloxycarbonyl (AcOZ) group which embodies an acetic acid ester as the enzyme-labile bond (Figure 4). Accordingly, the AcOZ group can be removed under conditions typical for acetyl ester hydrolysis, for instance by treatment with lipases or esterases.[53−55] As lipases display a broad specificity, other esters present in the substrate molecule might be hydrolyzed during AcOZ removal. Thus, the *p*-(phenylacetyl)benzyloxycarbonyl (PhAcOZ) group was developed which takes advantage of the high selectivity of penicillin G acylase for the phenylacetyl group (Figure 4). The versatiliy of this enzyme-labile urethane protecting group was demonstrated in the synthesis of phosphorylated,[56−60] glycosylated,[56−60] and lipidated[61] peptides.

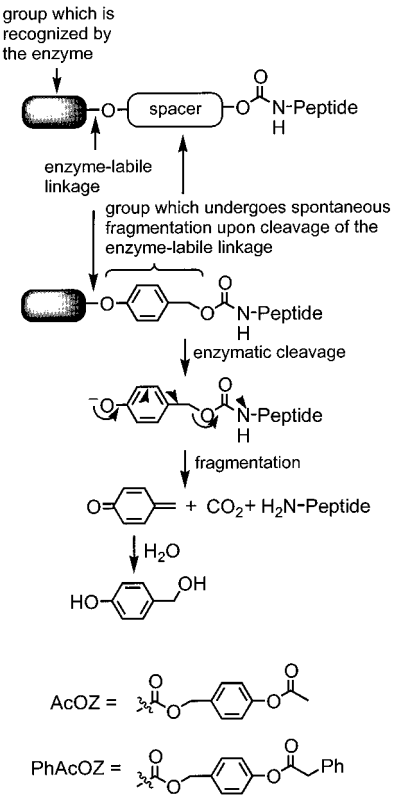



**Figure 4.** Principle of the spacer-based protecting groups AcOZ and PhAcOZ.

A second approach takes advantage of a characteristic property of glycosidases. It is well-known that glycosidases hydrolyze their substrates by cleaving the glycosidic bond via nucleophilic attack at the anomeric carbon atom. Therefore, a carbohydrate-derived urethane protecting group would provide the desired enzyme lability. Additionally, such sugar derivatives have increased solubility in aqueous solutions, a necessary requirement for all biotransformations. This concept was successfully realized by using glucose and galactose as the carbohydrate component (Figure 5).[62,63] During the synthesis the carbohydrate hydroxy functions are blocked by either benzyl ethers in the tetra-*O*-benzyl-D-glucopyranosyloxycarbonyl (BGloc) group or acetyl groups in the tetra-*O*-acetyl-D-glucopyranosyloxycarbonyl (AGloc) or the tetra-*O*-acetyl-*β*-D-galactopyranosyloxycarbonyl (AGaloc) protecting groups. The removal of these carbohydrate-based protecting groups proceeds via a two-step process by removing the hydroxyl blocking function in a first step followed by treatment with a glucosidase (AGloc, BGloc) or galactosidase (AGaloc), respectively. In the case of the acetyl derivatives AGloc and AGaloc, a sequential two-step process as well as a one-pot procedure were developed for the deprotection reaction, allowing for a convenient deprotection protocol as demonstrated for dipeptide **11** (Figure 5).[62]

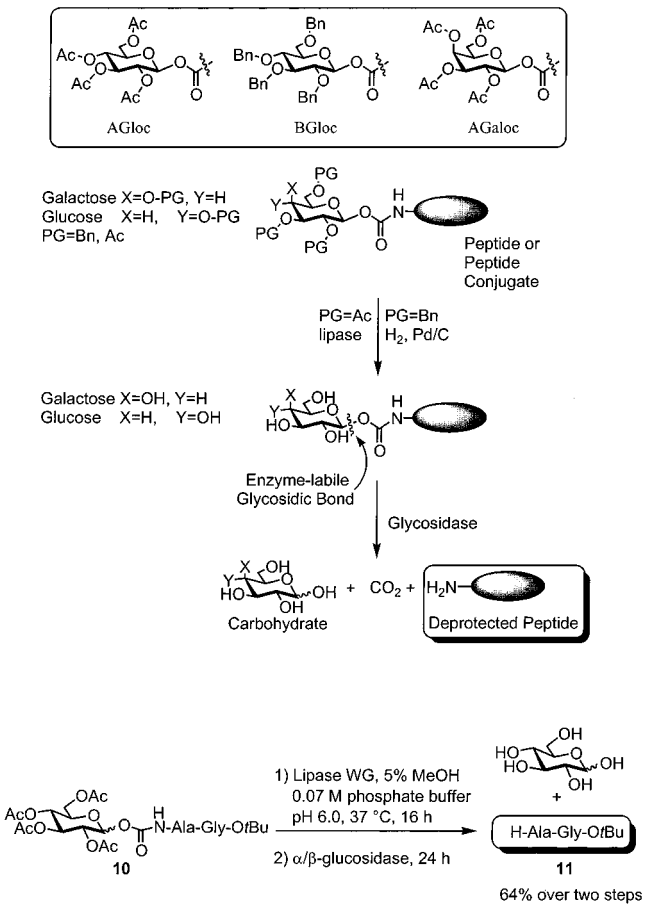



**Figure 5.** Carbohydrate-based urethane protecting groups.

## C. Protection of the Side Chain Amino Group of Lysine

During chemical peptide syntheses and if trypsin is used for the construction of the peptide bonds or N-terminal deprotection, the side chain amino group of lysine generally has to be protected to prevent side reactions.[13,14] This goal can be achieved enzymatically by applying the penicillin G acylase-catalyzed removal of the PhAc group (vide supra).[64] Thus, the first application of the PhAc group in peptide chemistry consisted in a synthesis of 1-deamino-Lys$^8$-vasopressin from the protected congener **9**, during which the lysine side chain was masked as the phenylacetamide (Figure 3). After the peptide chain had been assembled and the disulfide bond was formed by oxidative cyclization, the PhAc group could be removed enzymatically in 74% yield without side reaction. A further interesting example which demonstrates that this technique can advantageously be applied to the synthesis of even larger peptides is found in the complete deprotection of $(PhAc)_3$porcine insulin (vide supra, Figure 3)[27] and modified insulin fragments.[65] Since penicillin acylase is commercially available and devoid of peptidase activity,[66] this method appears to be generally useful for the construction of lysine-containing oligopeptides.

In addition to the PhAc group, pyroglutamyl amides (Glp) were proposed as enzymatically removable blocking functions for the lysine side chain.[23] Their removal was achieved with pyroglutamate aminopeptidase from calf liver. Thus, all N-protecting groups were split off the protected RNAse 1−10 fragment Glp-Lys(Glp)-Glu-Thr-Ala-Ala-Ala-Lys(Glp)-Phe-Glu-Arg-OH and from a model dipeptide. The general usefulness of this method remains to be demonstrated, however.

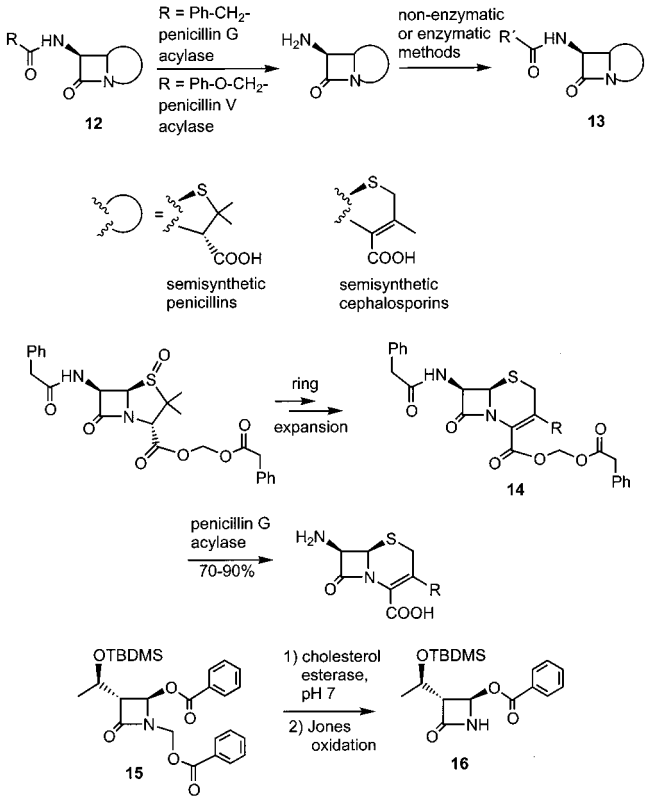



**Figure 6.** Enzymatic deprotection of amino and carboxy groups in $\beta$-lactam chemistry.

## D. Protection of Amino Groups in $\beta$-Lactam Chemistry

The enzymatic removal of acyl groups plays an important role in the industrial production of semisynthetic penicillins and cephalosporins. To this end, penicillin G **12** (R = $CH_2$−Ph) and penicillin V **12** (R = $CH_2$−O−Ph) or the respective cephalosporins are first deacylated by means of penicillin acylases (Figure 6).[67,68] The 6-aminopenicillanic acid and the 7-aminocephalosporanic acid thus obtained are subsequently acylated by nonenzymatic or enzymatic methods to give the semisynthetic antibiotics **13**.

The manufacturing of therapeutically important cephalosporins from penicillin G and V includes a chemical ring expansion of the thiazolidine ring to a dihydrothiazine. In the course of this sequence the amino group remains protected as phenylacetyl or phenoxyacetyl amide which is finally removed using penicillin G or V acylase. Of particular importance is the choice of a suitable protecting function for the COOH group. It must be stable during the ring expansion but removable without damaging the ceph-3-em nucleus. As an alternative to chemical methods, the use of the phenylacetoxymethylene ester was suggested for this purpose.[41,69] It is easily introduced and is stable during the construction of the cephalosporin framework (Figure 6). Together with the phenylacetamide, the ester can finally be removed

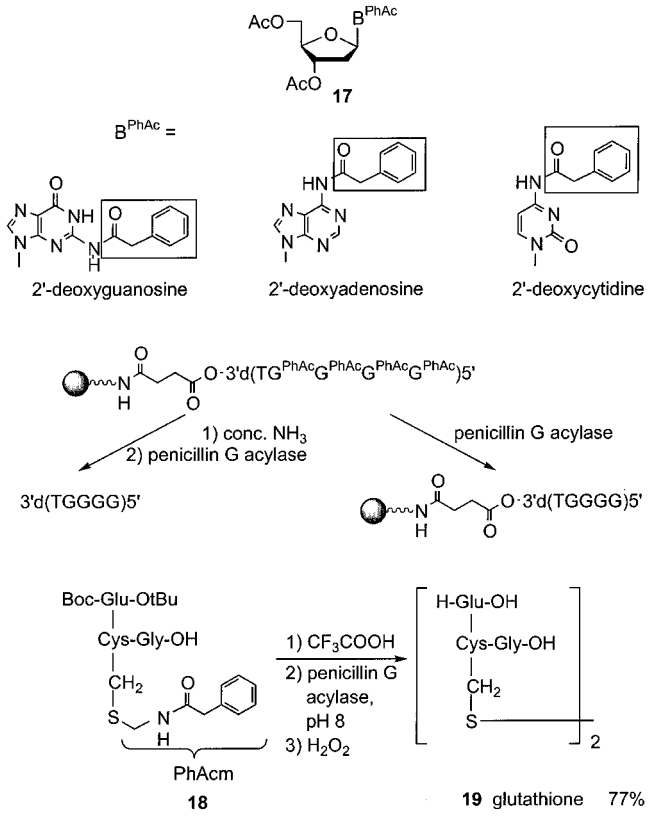



**Figure 7.** Enzymatic deprotection of the amino groups of nucleobases and the mercapto group of cysteine by means of penicillin G acylase. The shaded balls stand for controlled pore glass (CPG).

in high yield from penicillin G and the cephalosporins **14** by penicillin G acylase. The formaldehyde formed in the deprotection is not harmful to the enzyme.

In a new approach to well-known versatile $\beta$-lactam building blocks, an enzymatic deprotection of an acylated methylol amide was advantageously applied (Figure 6).[70] Thus, the dibenzoate **15** was regioselectively saponified by cholesterol esterase at pH 7, giving rise to a monoacylated aminal. After Jones oxidation and subsequent loss of formaldehyde, the azetidinone **16** was obtained which can be transformed into various enantiomerically pure penem and carbapenem building blocks.

As an alternative to the well-established phenylacetyl group in $\beta$-lactam chemistry, recently a biocatalyzed procedure for the phthalyl imide removal has been described (Figure 2).[45,71] Its general usefulness remains to be demonstrated, however.

## E. Protection of Amino Groups of Nucleobases

The amino groups of the nucleobases adenine, guanine, and cytosine in general must be protected during oligonucleotide synthesis to prevent undesired side reactions. To this end, they usually are converted into amides which finally are hydrolyzed under fairly basic conditions. If the amino functions are, however, masked as phenylacetamides, the protecting functions can be cleaved off by employing again penicillin G acylase (Figure 7).[72−78] The enzyme, for instance, selectively liberates the amino groups of the deoxynucleosides **17** without attacking the acetates in the carbohydrate parts and without damage of the acid-labile N-glycosidic bonds.

The biocatalyzed phenylacetyl removal can be carried out using both solubilized or immobilized substrates.[77] The latter methodology has been worked out using controlled pore glass (CPG) as a solid support (Figure 7).

# *III. Protection of Thiol Groups*[4−6,8,12]

## A. Protection of the Side Chain Thiol Group of Cysteine

The liberation of the $\beta$-mercapto group of cysteine was achieved by means of the penicillin G acylase-mediated hydrolysis of phenylacetamides also.[33−35] To this end, the SH group was masked with the phenylacetamidomethyl (PhAcm) blocking function (Figure 7). After penicillin acylase-catalyzed hydrolysis of the amide incorporated in the acylated thioaminal (see, e.g., **18**), a labile *S*-aminomethyl compound is formed which immediately liberates the desired thiol. This technique was, for instance, applied in a synthesis of glutathione, which was isolated as the disulfide **19**. In a related glutathione synthesis the method was used for the simultaneous liberation of the SH- and N-terminal amino function of glutamine.[34,35]

# *IV. Protection of Carboxy Groups*[4−9,12,79]

## A. C-Terminal Protection of Peptides

As in the enzymatic liberation of the N-terminus of peptides, initial attempts to achieve an enzyme-catalyzed deprotection of the corresponding carboxyl groups concentrated on the use of the endopeptidases chymotrypsin,[80−82] trypsin,[81,83,84] and thermolysin,[85] a protease obtained from *Bacillus thermoproteolyticus* which hydrolyzes peptide bonds on the amino side of hydrophobic amino acid residues (e.g., leucine, isoleucine, valine, phenylalanine). This latter biocatalyst served for the cleavage of the "supporting" tripeptide ester H-Leu-Gly-Gly-OEt from a protected undecapeptide (pH 7, room temperature). The octapeptide thereby obtained was composed exclusively of hydrophilic amino acids. Due to the broad substrate specificity of thermolysin and the resulting possibility of unspecific peptide hydrolysis, this method cannot be regarded as being generally applicable.

The exploitation of the esterase activities of chymotrypsin and trypsin opened routes to the hydrolysis of several peptide methyl, ethyl, and *tert*-butyl esters at pH 6.4−8 and room temperature.[80,81] Not only is the transformation successful with peptides carrying the respective enzyme-specific amino acids at the C-terminus, but in several cases different amino acids were tolerated at this position also. However, severe drawbacks of this methodology are that numerous peptides are poor substrates or not accepted at all. Moreover, a competitive cleavage of the peptide bonds occurs if the peptides contain trypsin- or chymotrypsin-labile sequences. Therefore, these proteases appear to be not generally useful for a safe C-terminal deprotection as well.

H–[Tyr]–Gly–Gly–[Phe]–Met–OH

**20** methionine enkephalin

□ = C-terminally deprotected by enzymatic saponification of the peptide amide with carboxypeptidase Y;Tyr was N-terminally deprotected by removal or Bz-Arg with trypsin

PG-peptide-$NH_2$ —(amidase from the *flavedo* of oranges, pH 7.5, 30°C)→ PG-peptide-OH

| PG | peptide | conv. [%] |
|---|---|---|
| Bz | Tyr-Ser | 100 |
| Boc | Leu-Val | 20 |
| Trt | Gly-Leu-Val | 100 |
| Z | Gly-Gly-Leu | 100 |

**Figure 8.** C-Terminal deprotection of peptide amides by carboxypeptidase Y and an amidase from the *flavedo* of oranges.

The disadvantages displayed by the endopeptidases can be overcome by using carboxypeptidase Y from Baker's yeast.[25,86,87] This serine-exopeptidase also has esterase activity and is characterized by quite different pH-optima for the peptidase and the esterase activity (pH > 8.5). Even in the presence of various organic cosolvents, the enzyme selectively removes the carboxy protecting groups from a variety of differently protected di- and oligopeptide methyl and ethyl esters[25,87] without attacking the peptide bonds. An additional attractive feature is that its esterase activity is restricted to α-esters; consequently, β- and γ-esters of aspartic and glutamic acid, respectively, are not attacked. Carboxypeptidase Y was advantageously used for the stepwise C-terminal elongation of the peptide chain in aqueous solution employing a solubilizing poly(ethylene glycol)-derived polymeric support as the N-terminal blocking group.[86] In a further remarkable synthesis which did not include the use of a polymeric N-protecting group, Met-enkephalin **20** was built up by employing carboxypeptidase Y for C-terminal deprotection of intermediary generated peptide amides as well as for the formation of the peptide bonds (Figure 8).[25]

The additional opportunity to selectively hydrolyze C-terminal peptide amides with carboxypeptidase Y is of particular interest if, as demonstrated in the abovementioned example, enzymatic methods are applied for the formation of the peptide bonds, because amino acid amides often are the nucleophiles of choice in these biocatalyzed processes. For this purpose a peptide amidase from the *flavedo* of oranges shows very promising properties.[88−90] The enzyme is equipped with a broad substrate specificity and accepts Boc-, Trt-, Z-, and Bz-protected and N-terminally unprotected peptide amides (Figure 8). The C-terminal amides are saponified in high yields at pH 7.5 and 30 °C without affecting the N-terminal blocking groups or the peptide bonds. A noticeable advantage of this biocatalyst is that N-deprotected amino acid amides, in contrast to the respective peptide amides, do not belong to its substrates. They can, therefore, be used as nucleophiles in peptide

PG-peptide-OR —(alkaline protease from *Bacillus subtilis* DY, pH 8, 37°C)→ PG-peptide-OH

| PG | peptide | R | yield [%] |
|---|---|---|---|
| Z | Tyr(tBu)-Glu-Leu | Me | 93 |
| Boc | Leu-Glu-Val | Bzl | 85 |
| Trt | Ala-Glu-Asp-Leu-Glu | Bzl | 80 |

PG-peptide-OR —(alcalase, pH 8.2, 35°C; 90 vol% tert-butanol, 10 vol% buffer)→ PG-peptide-OH

| PG | peptide | R | yield [%] |
|---|---|---|---|
| Fmoc | Ala-Val-Ile | Me | 85 |
| Fmoc | Asn-Phe | Bzl | 90 |
| Boc | Met-Leu-Phe | Me | 80 |
| Z | Met-Asp(OMe)-Phe | Me | 90 |

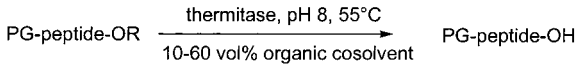
**Figure 9.** C-Terminal deprotection of peptide esters by the alkaline protease from *Bacillus subtilis DY* and alcalase.

syntheses catalyzed by this enzyme, i.e., the formation of the peptide bond together with the subsequent C-terminal deprotection is achieved in a single step.

A further possibility for the enzymatic removal of C-terminal blocking groups is opened up by the application of enzymes which generally display a high esterase/protease ratio. Such a biocatalyst is the alkaline protease from *Bacillus subtilis DY*, which shows similarities to Subtilisin Carlsberg. For this enzyme the ratio of esterase to protease activity is $>10^5$. It selectively removes methyl, ethyl, and benzyl esters from a variety of Trt-, Z-, and Boc-protected di- and tripeptides and a pentapeptide at pH 8 and 37 °C (Figure 9).[91]

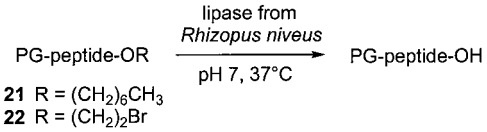
The N-terminal urethanes and the peptide linkages are left intact. A further protease which fulfills the requirements for a successful application in peptide chemistry is alcalase, a serine endopeptidase from *Bacillus licheniformis* whose major component is subtilisin A (Subtilisin Carlsberg).[92−94] It can advantageously be employed to selectively saponify peptide methyl and benzyl esters (Figure 9). In a solvent system consisting of 90% *tert*-butyl alcohol and 10% buffer (pH 8.2), even highly hydrophobic and in aqueous solution insoluble Fmoc peptides were accepted as substrates and deprotected at the C-terminus without any disturbing side reaction. A selective classical alkaline saponification of methyl esters would be impossible due to the base-sensitivity of the Fmoc group.

A very promising and unusually stable biocatalyst is thermitase, a thermostable extracellular serine protease from the thermophilic microorganism *Thermoactinomyces vulgaris* whose esterase/protease ratio amounts to >1000:1. The enzyme shows a broad amino acid side chain specificity and cleaves methyl, ethyl, benzyl, methoxybenzyl-, and *tert*-butyl esters from a variety of Nps-, Boc-, Bpoc-, and Z-protected di- and oligopeptides in high yields at pH 8 and 35−55 °C (Figure 10).[33,34,95−97] In addition, it is specific

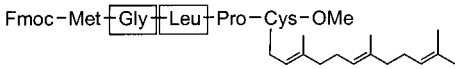



| PG | peptide | R | yield [%] |
|---|---|---|---|
| Z | Leu-Val-Glu(tBu)-Ala | Me | 92 |
| Boc | Pro-Gly | Me | 73 |
| Bpoc | Tyr(tBu)-Glu-Leu | Me | 55 |
| Nps | Ser(Bzl)-His(Dnp)-Leu-Val-Glu(tBu)-Ala | Me | 90 |

**Figure 10.** C-Terminal deprotection of peptide esters by thermitase.




| PG | peptide | R | yield [%] |
|---|---|---|---|
| Boc | Ser-Thr | Hep | 95 |
| Z | Thr-Ala | Hep | 85 |
| Aloc | Met-Gly | Hep | 90 |
| Z | Ser-Phe | EtBr | 84 |
| Boc | Val-Ala | EtBr | 95 |




**23** C-terminal pentapeptide of the N-Ras protein

□ = C-terminally deprotected by employing lipase from *Rhizopus niveus*

**Figure 11.** C-Terminal deprotection of peptide esters by lipase from *Rhizopus niveus*.

for the α-carboxy groups of Asp and Glu. To enhance the solubility of the substrates, up to 50 vol % of organic cosolvents such as DMF and DMSO may be added, which also serve to reduce the remaining peptidase activity to a negligible amount.[34,97]

In the discussion of the protease-catalyzed cleavage of the N-terminal protecting groups it was already pointed out that the use of biocatalysts belonging to this class of enzymes in general, i.e., also for the C-terminal deblocking, may lead to an undesired hydrolysis of peptide bonds. In particular, this has to be expected if the respective ester or amide to be hydrolyzed turns out to be only a poor substrate which is only attacked slowly, an experience not uncommon if unnatural substrates are subjected to enzyme-mediated transformations. This undesired possibility would, however, be overcome if enzymes were used which are not able to split amides at all. This principle has been realized in the development of the heptyl (Hep),[4−9,31,32,98−100] 2-bromoethyl (EtBr)[4−6,31,32,101] and *p*-nitrobenzyl (PNB) esters[102] as carboxy protecting groups for peptide synthesis which can be enzymatically removed by means of lipases or esterases, respectively (Figure 11).

The Hep-esters prove to be chemically stable during the removal of the N-terminal Z, Boc, and Aloc group from the dipeptides **21**. The selective removal of the Hep-esters is achieved by a lipase-catalyzed hydrolysis. From several enzymes investigated, a biocatalyst isolated from the fungus *Rhizopus niveus* was superior to the others with respect to substrate tolerance and reaction rate. The enzyme accepts a variety of Boc-, Z-, and Aloc-protected dipeptide Hep-esters as substrates and hydrolyzes the ester functions in high yields at pH 7 and 37 °C without damaging the urethane protecting groups and the amide bonds (Figure 11).[98,99] Z- and Boc-dipeptide-2-bromoethyl esters **22** are attacked also with comparable or in some cases even higher rates. In the presence of either one of the enzyme-labile protecting groups, the N- and C-terminal amino acid can be varied in a wide range. With increasing steric bulk and lipophilicity of the amino acids, especially the C-terminal one, the rate of the enzymatic reactions decreases. If the C-terminal amino acid is proline, the enzymatic reaction does not take place. The lipase-mediated deprotection of peptides was, for instance, successfully applied in the construction of the C-terminal pentapeptide methyl ester **23** of the N-Ras-protein, which is localized in the plasma membrane and plays a vital role in cellular signal transduction (Figure 11).[103]

The use of lipases for the removal of protecting groups from peptides in addition to the absence of protease activity has several advantages. Various enzymes belonging to this class and stemming from different natural sources (including mammals, bacteria, fungi, and thermophilic organisms) are commercially available and rather inexpensive. This variety gives the opportunity to replace a chosen biocatalyst by a better one if a particular substrate is only attacked slowly (vide infra). The lipases are not specific for L-amino acids but also tolerate the presence of the D-enantiomer.[104] A noticeable feature is that in contrast to proteases and esterases, they operate at the interface between water and organic solvents.[105] This is particularly important if longer peptides being composed of hydrophobic amino acids and/or carrying side chain protecting groups and which do not dissolve well in the aqueous systems have to be constructed.

The full capacity of the lipase-mediated technique for C-terminal deprotection was demonstrated in the synthesis of complex base-labile phosphopeptides[44] and *O*-glycopeptides which are sensitive to both acids and bases.[106,107] To this end, e.g., the serine glycoside **24** was selectively deprotected at the C-terminus by lipase from the fungus *Mucor javanicus* (Figure 12).

The carboxylic acid **25** liberated thereby was then coupled with an N-terminally deprotected glycodipeptide, and after subsequent enzyme-mediated deprotection, the glycotripeptide carboxylic acid **26** was obtained in high yield. This compound was finally condensed with a tripeptide to give the complex diglycohexapeptide **27**, which carries the characteristic linkage region of a tumor-associated glycoprotein antigen found on the surface of human breast cancer cells. In the course of these enzymatic transformations, the N-terminal urethanes, the peptide bonds, the acid- and base-labile glycosidic linkages, and the acetyl protecting groups which are sensitive to bases were not attacked. In these cases, lipase from *Rhizopus niveus*, which was the enzyme

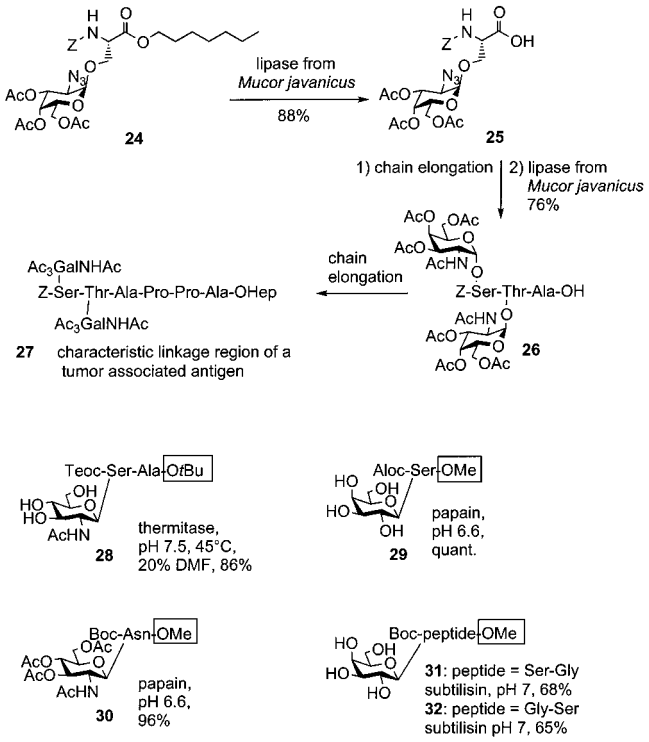



**Figure 12.** Construction of acid- and base-labile glycopeptides via enzyme-mediated C-terminal deprotection.

of choice for simple peptides, attacked the substrates very slowly so that a different biocatalyst had to be used. This demonstrates the abovementioned advantage of being able to apply several catalytic proteins of comparable activity but different substrate tolerance for the solution of a given synthetic problem.

The viability and the wide applicability of the principle to use enzymes for the removal of individual protecting groups from complex multifunctional compounds such as lipo- and glycopeptides is further proven by the finding that proteases can be used for this purpose also. Thus, by means of thermitase-catalysis, the C-terminal *tert*-butyl ester was removed from the glycopeptide **28** (Figure 12).[34,108] In a different study, this enzyme was also used for the cleavage of methyl and *p*-nitrobenzyl esters.[109] From the serine glycoside **29**[110,111] and from the asparagine conjugate **30**,[112] the methyl esters could be cleaved off without disturbing the side reaction by using papain as the biocatalyst. Similarly, the liberation of the C-terminal carboxy group of the glycosylated dipeptides **31** and **32** was achieved by means of subtilisin-catalyzed hydrolysis.[113] However, in these cases, papain could not be used since this protease preferably cleaved the peptide bonds. This example highlights again the danger associated with the use of a protease for the removal of protecting groups from peptides.

A regularly arising problem in enzymatic deprotection is the poor solubility of the fully blocked peptides in the required aqueous media, resulting in a limited accessibility of the substrates to the enzymes. To overcome this difficulty, in many cases solubilizing organic cosolvents are added; however, a more general and viable approach consists of the introduction of solubilizing protecting groups, e.g., in

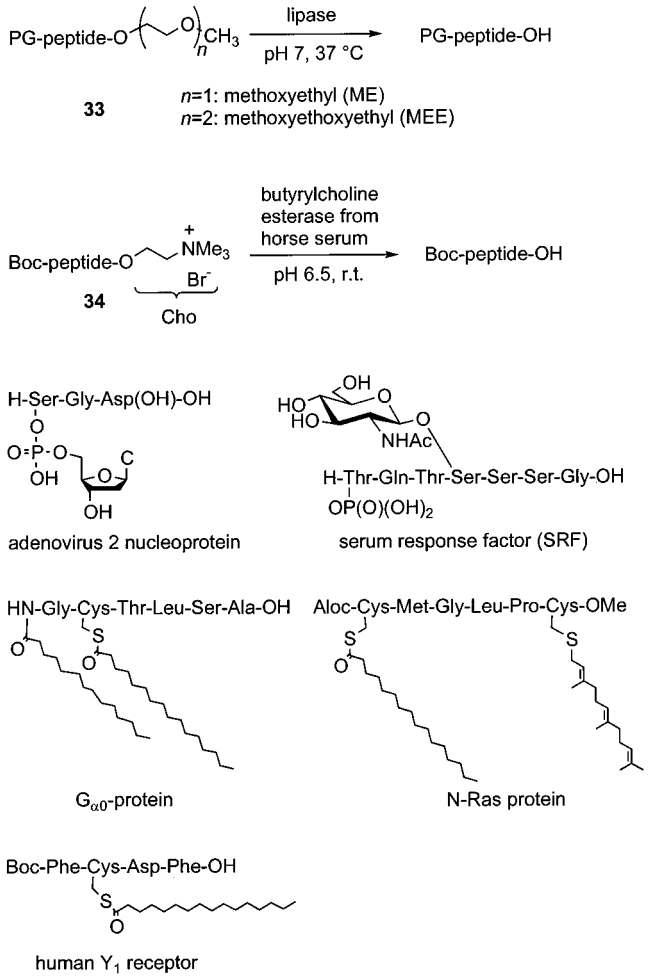



**Figure 13.** Use of hydrophilic esters as solubilizing enzymatically removable protecting groups for the synthesis of characteristic protein fragments.

the enzyme-mediated formation of peptide bonds.[114] An enzymatically removable solubilizing ester protecting group could be found in the ethylene glycol-derived esters such as the methoxyethyl (ME) esters[78,115] and the methoxyethoxyethyl (MEE) esters[78,115–117] and in the choline esters (Figure 13).[58,59,76,78,118–121] The ME and MEE esters serve as hydrophilic analogues of the heptyl esters discussed above and can be therefore removed by the same biocatalysts, such as the lipase from *Mucor javanicus*. Their increased solubility in aqueous media has been successfully used in the synthesis of small peptides and peptide conjugates including glyco-[115–117] and nucleopeptides.[78]

Similarly, the respective dipeptide choline esters **34** are easily soluble in purely aqueous media (i.e., without added cosolvent) and are converted into the corresponding carboxylic acids under the mildest conditions and without side attack on the peptide bonds and the N-terminal urethanes by means of the commercially available butyrylcholine esterase from horse serum. The increased hydrophilicity of peptide choline esters was advantageously used for the synthesis of peptides and very sensitive peptide conjugates such as lipidated peptides,[118–121] phosphorylated and glycosylated peptides,[58,59] and nucleopeptides (Figure 13).[76,78]

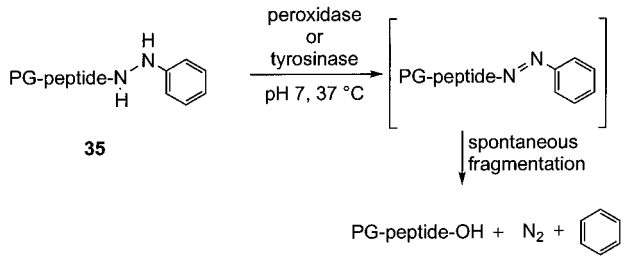



**Figure 14.** Phenylhydrazide as a carboxy protecting group.

Recently the phenylhydrazide has been introduced as an enzyme-labile carboxy protecting group.[122,123] This protecting group can be removed by mild enzymatic oxidation using an peroxidase[122,123] or mushroom tyrosinase[124] (Figure 14).

## B. Protection of the Side Chain Groups of Glutamic and Aspartic Acid

The stepwise removal of arginine methyl ester by proteases has been investigated as a possibility for the enzymatic deprotection of the side chain carboxylate groups of the aminodicarboxylic acids aspartic acid (Asp) and glutamic acid (Glu). To this end, Z-Asp(ArgOMe)-$NH_2$ and Z-Glu(ArgOMe)-$NH_2$ were converted to Z-Asp(OH)-$NH_2$ and Z-Glu(OH)-$NH_2$ by subsequent treatment with trypsin, which hydrolyzes the arginine methyl esters, and with porcine pancreatic carboxypeptidase B, which splits off the arginines.[125] Since the second step is slow and requires high concentrations of the carboxypeptidase, this method most likely cannot be routinely applied in peptide synthesis because it introduces too much danger of competitive side reactions.

However, enzymatic transformations have proven to be useful for the synthesis of selectively functionalized aspartic and glutamic acid derivatives. For instance, alcalase selectively hydrolyzes the α-benzyl esters of H−Asp(Bzl)-OBzl and H-Glu(Bzl)-OBzl in 82% and 85% yields, respectively, on a decagram scale.[126] Similarly, aspartyl- and glutamylpeptides can be deprotected selectively at the C-terminus by this enzyme; however, in these cases an undesired attack on the peptide bonds may occur.[127] In addition, Z-Asp(OAll)-OAll is converted to Z-Asp(OAll)-OH in quantitative yield by papain.[128] Also, a lipase from *Candida cylindracea* is able to differentiate between the two carboxylic acid groups of glutamic acid. From the respective dicyclopentyl ester it preferably (ratio 20:1) removes the γ-ester in 90% yield.[129] In addition, the enzyme thermitase and the alkaline protease from *Bacillus subtilis* (vide supra) also hold a great potential for the selective manipulation of dicarboxylic amino acids.

The examples given in sections II and III demonstrate that the selective deprotection of peptides can be achieved advantageously by making use of enzymatic reactions. In light of the increasing number of available biocatalysts, it appears that soon a host of new and superior enzymatically removable blocking groups for the synthesis of peptides will be developed. However, these techniques will certainly not be used for the preparation of simple small peptides in the laboratory. Most likely they will be applied for the synthesis of sensitive polyfunctional compounds and long oligopeptides whose construction is cumbersome by standard chemical methods. Furthermore, they offer significant advantages if a technical process for the manufacturing of a given peptide has to be developed. Finally, together with the recently developed methods for the biocatalyzed formation of peptide bonds,[130] enzymatic protecting group techniques could prove to be the tools of choice for the construction of peptides in aqueous solution, which one has tried to develop to practicability for several decades.[131,132]

## V. Protection of Hydroxy Groups[4−9,133−136]

Mono- and oligosaccharides, alkyl- and arylglycosides, and various other glycoconjugates generally embody a multitude of hydroxyl groups of comparable chemical reactivity. Also, the synthesis of oligonucleotides and nucleosides, β-lactams, alkaloids, steroids, and peptides often requires the selective protection of one or more alcoholic functions. Consequently, for the directed construction of polyhydroxy compounds, these functional groups have to be manipulated selectively, in general making cumbersome protection and deprotection steps necessary. Although numerous chemical techniques are available to mask or liberate hydroxyl groups,[1−3] the development of enzymatic methods for this purpose has been progressing steadily and appears to complement the arsenal of classical tools. Additionally, the enzymatic protection of hydroxy goups (and vice versa of carboxy groups) in racemic compounds as well as their enzyme-catalyzed deprotection has been extensively used for the separation of enantiomeric alcohols and carboxylic acids.

## A. Protection of Monosaccharides[133,137]

The selective protection and deprotection of carbohydrates can be achieved with various classical chemical techniques.[1−3,138−140] In addition, however, due to the synthetic challenge the multifunctional carbohydrates pose, enzymatic techniques for the introduction of blocking groups into sugars and/or their subsequent removal offer further different opportunities.

The enzymatic acylation of sugars in aqueous solution has been reported but gives low yields as the equilibrium for the reaction favors hydrolysis. However, enzymatic acylation in dry organic solvents has shown substantial success. While direct enzymatic esterification of alcohols with acids is often not practical, good to excellent yields have been obtained using transesterification techniques (Table 1). The displacement of the equilibrium toward products has been accomplished by using an excess of the acyl donor and activated, irreversible acyl donors such as trihaloethyl esters,[141] enol esters,[142] acid anhydrides, or oxime esters.[134,136] In particular, the enol esters have the advantage that the liberated enol tautomerizes to a ketone or an aldehyde, thereby shifting the equilibrium toward the desired products and consequently giving higher yields. This technology, how-

**Table 1. Selective Acylation of the Primary Hydroxy Group in Monosaccharides**

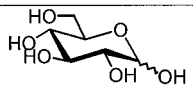
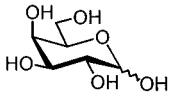
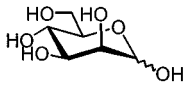
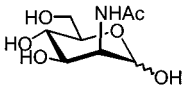
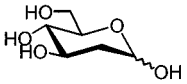
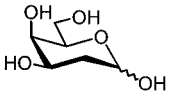
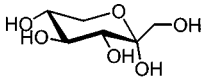
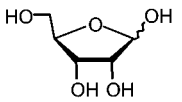
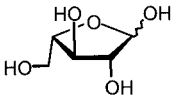
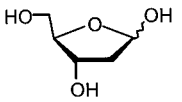
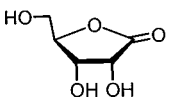
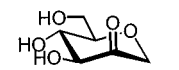
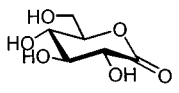
| Compound No. Structure | Enzyme[a] | Solvent | Acyl Donor | Position | Yield (%) | Ref. |
|---|---|---|---|---|---|---|
| **36**  | PPL | pyridine | $RCO_2CH_2CCl_3$ | 6 | 19-35 | [141] |
| | CAL | dioxane | $ROCO_2N{=}CMe_2$ | 6 | 15-72 | [145] |
| | CAL | THF | $RCO_2CH{=}CH_2$ | 6 | | [147] |
| | PSL | pyridine | $MeCO_2CH_2CCl_3$ | 6 | 79 | [146] |
| | PSL | pyridine | $EtCO_2CH_2CCl_3$ | 6 | 29 | [146] |
| | proleather | pyridine | $PhCO_2CH_2CCl_3$ | 6 | 33 | [146] |
| | subtilisin | DMF | $PrCO_2CH_2CCl_3$ | 6 | 60 | [150] |
| | subtilisin | pyridine | $PrCO_2CH_2CCl_3$ | 6 | 64 | [150] |
| | optimase M-440 | pyridine | Boc-Phe-$OCH_2CF_3$ | 6 | | [151] |
| **37**  | PPL | pyridine | $MeCO_2CH_2CCl_3$ | 6 | 57 | [141] |
| | PSL | pyridine | $RCO_2N{=}CMe_2$ | 6 | 70-85 | [152] |
| | CAL | dioxane | $ROCO_2N{=}CMe_2$ | 6 | 43-68 | [145] |
| **38**  | PPL | pyridine | $MeCO_2CH_2CCl_3$ | 6 | 36 | [141] |
| | CCL | benzene/pyridine 2:1 | $MeCO_2CH{=}CH_2$ | 6 | | [142] |
| | PSL | pyridine | $RCO_2N{=}CMe_2$ | 6 | 65-80 | [152] |
| | CAL | dioxane | $ROCO_2N{=}CMe_2$ | 6 | 44-53 | [145] |
| | protease N | DMF | $MeCO_2C(Me){=}CH_2$ | 6 | 40 | [153] |
| **39**  | CCL | benzene/pyridine 2:1 | $MeCO_2C(Me){=}CH_2$ | 6 | | [142] |
| | protease N | DMF | $MeCO_2C(Me){=}CH_2$ | 6 | 73 | [153] |
| | subtilisin 8399 | DMF | $MeCO_2CH{=}CH_2$ | 6 | 92 | [154] |
| | subtilisin BNP' | 97 % DMF | Boc-Gly-$OCH_2CN$ | 6 | 65 | [155] |
| **40**  | CAL | pyridine | $RCO_2N{=}CMe_2$ | 6 | 45-83 | [156] |
| | PSL | dioxane | $RCO_2N{=}CMe_2$ | 6 | 50-72 | [156] |
| **41**  | CAL | pyridine | $RCO_2N{=}CMe_2$ | 6 | 57-81 | [156] |
| | PSL | dioxane | $RCO_2N{=}CMe_2$ | 6 | 47-62 | [156] |
| **42**  | PSL | pyridine | $RCO_2N{=}CMe_2$ | 1 | 68-86 | [152] |
| **43**  | CAL | pyridine | $RCO_2N{=}CMe_2$ | 5 | 50-64 | [152] |
| | CAL | dioxane | $ROCO_2N{=}CMe_2$ | 5 | 37-52 | [145] |
| | CAL | THF | $Pr_2O$ | 5 | | [157,158] |
| **44**  | CAL | pyridine | $RCO_2N{=}CMe_2$ | 5 | 45-70 | [152] |
| | CAL | dioxane | $ROCO_2N{=}CMe_2$ | 5 | 38-49 | [145] |
| **45**  | CAL | THF | $Pr_2O$ | 5 | | [157,158] |
| **46**  | PPL | pyridine | $C_{11}H_{23}CO_2CH_2CCl_3$ | 5 | 40 | [159] |
| **47a**  | CAL | acetone/pyridine 3:1 | $C_{11}H_{23}CO_2H$ | 6 | 67 | [160] |
| **47b**  | CAL | *t*BuOH | $C_{11}H_{23}CO_2Et$ | 6 | 51 | [161] |

**Table 1 (Continued)**

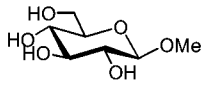
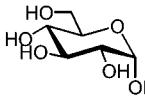
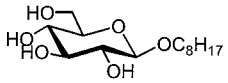
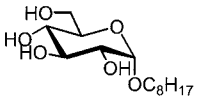
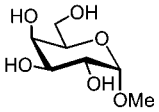
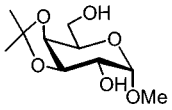
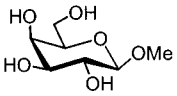
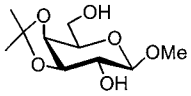
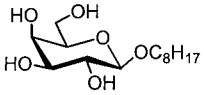
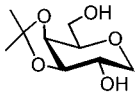
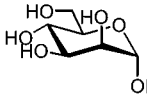
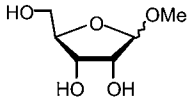
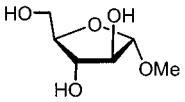
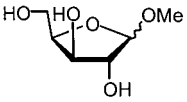
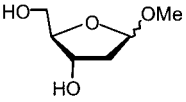
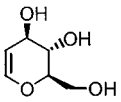
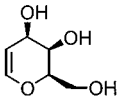
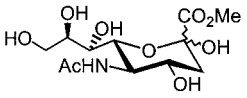
| Compound No. Structure | Enzyme[a] | Solvent | Acyl Donor | Position | Yield (%) | Ref. |
|---|---|---|---|---|---|---|
| **48**  | CCL | benzene/pyridine 2:1 | $MeCO_2CH=CH_2$ | 6 | | [142] |
| | CAL | THF/pyridine (4:1) | $MeCO_2CH=CH_2$ | 3,6 | | [162] |
| | CAL | $PrCO_2Et$/*t*BuOH (1:1) | $PrCO_2Et$ | 6 | | [148,149] |
| **49**  | CAL | $CH_2=CHCO_2Et$/ *t*BuOH (1:1) | $CH_2=CHCO_2Et$ | 6 | | [149] |
| | CAL | THF/pyridine (4:1) | $MeCO_2CH=CH_2$ | 6 | | [162] |
| **50**  | CAL | THF | $MeCO_2CH=CH_2$ | 6 | | [162] |
| | CAL | $PrCO_2Et$/*t*BuOH (1:1) | $PrCO_2Et$ | 6 | | [148,149] |
| | CAL | *t*BuOH | $Ph(CH_2)_3CO_2H$ | 6 | 52 | [163] |
| | CVL | THF | $PrCO_2CH_2CCl_3$ | 6; 3,6 (1:1) | | [164] |
| | ANL | THF | $PrCO_2CH_2CCl_3$ | 6; 3,6 (10:1) | | [164] |
| **51**  | CAL | $CH_2=CHCO_2Et$ | $CH_2=CHCO_2Et$ | 6 | | [149] |
| **52**  | PPL | pyridine | $PrCO_2CH_2CF_3$ | 6 | 79 | [165] |
| | CAL | THF/pyridine (4:1) | $MeCO_2CH=CH_2$ | 6; 3,6 (3:1) | | [162] |
| | CAL | $CH_2=CHCO_2Et$/ *t*BuOH (1:1) | $CH_2=CHCO_2Et$ | 2,6 | | [148,149] |
| **53**  | PSL | $MeCO_2CH=CH_2$/THF | $MeCO_2CH=CH_2$ | 6 | 93 | [166] |
| **54**  | CAL | THF/pyridine (4:1) | $MeCO_2CH=CH_2$ | 6; 2,6; 3,6 (1:1.3:1.8) | | [162] |
| | CAL | $CH_2=CHCO_2Et$/ *t*BuOH (1:1) | $CH_2=CHCO_2Et$ | 6; 2,6; 3,6 (2:1:1) | | [148,149] |
| **55**  | PSL | $MeCO_2CH=CH_2$/THF | $MeCO_2CH=CH_2$ | 6 | 90 | [166] |
| **56**  | PSL | $MeCO_2CH=CH_2$ | $MeCO_2CH=CH_2$ | 6 | 75 | [167] |
| **57**  | PSL | $MeCO_2CH=CH_2$/THF | $MeCO_2CH=CH_2$ | 6 | 94 | [166] |
| **58**  | PPL | pyridine | $PrCO_2CH_2CF_3$ | 6 | 81 | [165] |
| **59**  | PPL | THF | $MeCO_2CH_2CF_3$ | 5 | 77 | [168] |

**Table 1 (Continued)**

| Compound No. Structure | Enzyme[a] | Solvent | Acyl Donor | Position | Yield (%) | Ref. |
|---|---|---|---|---|---|---|
| **60**  | PPL | THF | $MeCO_2CH_2CF_3$ | 5 | 77 | [168] |
| **61**  | PPL | THF | $MeCO_2CH_2CF_3$ | 5 | 84 | [168] |
| **62**  | PPL | THF | $MeCO_2CH_2CF_3$ | 5<br>3 | 39<br>17 | [168] |
| **63**  | CCL | EtOAc | $MeCO_2CH{=}CH_2$ | 6 | 90 | [169] |
| **64**  | CCL | EtOAc | $MeCO_2CH{=}CH_2$ | 6 | 93 | [169] |
| **65**  | CCL | THF | $MeCO_2CH{=}CH_2$ | 9 | 60 | [170] |

[a] Usually many enzymes were screened for activity, only the best results are listed. CAL, *Candida antarctica* lipase; CCL, lipase from *Candida cylindracea* (later renamed *Candida rugosa*); PPL, porcine pancreas lipase; PSL, *Pseudomonas cepacia* lipase.

ever, is not restricted to carboxylic acid derivatives as acyl donor. Organic carbonates,[143] either activated as the vinyl[144] or—even better—as oxime[145] derivative, allow for the enzyme-catalyzed synthesis of carbonates such as the methoxycarbonyl, benzyloxycarbonyl (Z), and allyloxycarbonyl (Aloc) carbonate. The latter two examples can later be removed by nonenzymatic means.

The high polarity of sugars and their derivatives requires that polar solvents be used to dissolve them. Solvents found to be suitable include pyridine, DMSO, DMF, and dimethylacetamide. However, these solvents also often inactivate enzymes, although some enzymes, for instance, the lipases from the porcine pancreas (PPL), from *Candida antarctica* (CAL), from *C. cylindracea* (CCL, later renamed *Candida rugosa*), and the lipase from *Pseudomonas cepacia* (PSL) as well as the proteases subtilisin and Proleather, maintain their inherent acitvity.[146] A less polar solvent like THF allows the use of a broader variety of lipases but do not dissolve unmodified pyranoses. Nevertheless, it should be noted that even glucose suspended in THF has been successfully acylated by using lipase of *Candida antarctica*.[147]

To remain active in organic solvent, the enzyme must maintain a small amount of water which is required for maintaining the correct protein structure. In the absence of this essential water, highly polar compounds such as carbohydrates form excessively tight enzyme−product complexes. This inhibits association and dissociation of substrates and products from the active site and thus slows the reaction. Accordingly, the addition of drying agents such as zeolite CaA influences not only the biocatalyst's activity but also its selectivity. For instance, the acylation of 1-*O*-methyl $\beta$-D-glycopyranoside **49** catalyzed by lipase SP 435 (an immobilized lipase from *Candida antarctica*) in ethyl butanoate as solvent and acyl donor led to the predominant acylation in the 6-position.[148,149] If zeolite CaA was added, a mixture of 2,6- and 3,6-bisacylated pyranosides (95:5) was formed. In the presence of zeolite CaA and *tert*-butyl alcohol as a cosolvent, again monoacylation in the 6-position was observed.

Alternatively, precipitation of the enzyme from aqueous solution at its optimum pH prior to its use in organic solvent has also been reported to greatly increase the enzyme's activity.

The results of enzymatic acylation of several pyranose and furanose sugars are shown in Table 1. More lipophilic carbohydrate derivatives such as alkyl glycosides display a higher solubility in less polar organic solvents, in which most lipases tend to be more stable than in polar solvents.

A further interesting finding is that heat-stable lipases are capable of transferring long-chain fatty acids to the 6-hydroxyl group of ethyl glucoside on a kilogram scale using the molten fatty acids themselves as solvents.[171] On a somewhat smaller scale, the acylation of glucose has also been carried out using only a minute amount of solvent[172] or in supercritical $CO_2$.[173,174]

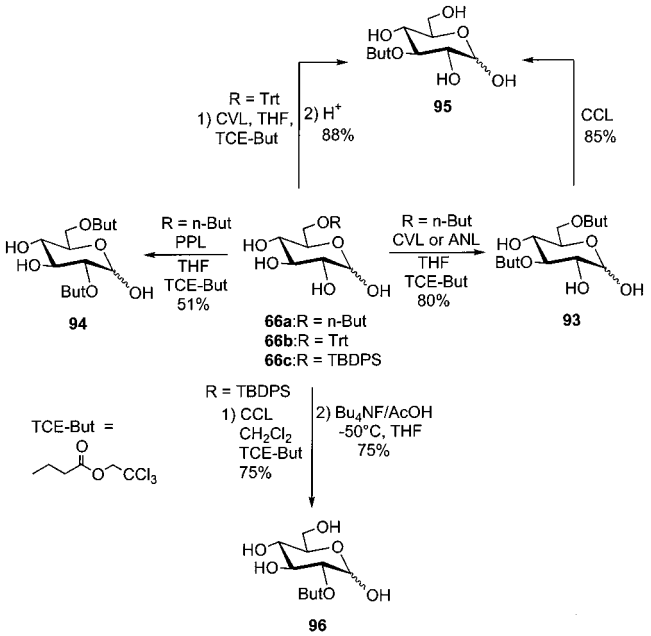



**Figure 15.** Selective enzymatic introduction of protecting groups into partially acylated hexoses.

The regioselectivity observed in the acylation of underivatized pyranoses in principle parallels that recorded for the classical chemical introduction of acyl groups into carbohydrates. However, if the 6-OH groups are protected first or deoxygenated, in the corresponding enzymatic reactions selectivities are observed which cannot be realized with classical chemical methods. By careful choice of solvent and lipase, it is possible to selectively modify a number of C-6-protected pyranoses at the secondary hydroxy groups (Table 2).

By combination of enzymatic with nonenzymatic protection group chemistry, carbohydrates can be selectively modified in the primary and secondary hydroxy positions. To demonstrate this versatility, the straightforward synthesis of differently monoacylated glucose derivatives is described in Figure 15. For instance, 6-*O*-butyrylated glucose **66a** (R = *n*-butanoyl; prepared enzymatically, see Table 1) is converted to the 3,6-dibutanoate **93** by lipase from *Chromobacterium viscosum* (CVL) or *Aspergillus niger* (ANL). The 2,6-dibutanoate **94** can conveniently be built up with the lipase from porcine pancreas (PPL; Figure 15).[164] Similar observations were reported for *n*-octylglucoside, but for the corresponding galactose- and mannose 6-esters, the selectivity was lower. In contrast, the chemical butyrylation of glucose derivative **66a** with the acid anhydride in pyridine gave a complex mixture of various diesters without remarkable regiodiscrimination. The enzymatic approach was also used to convert the 6-*O*-tritylglucose **66b** (R = Trt) to the 3-butanoate **95** by a chemoenzymatical approach with lipase from *C. viscosum* (CVL), and the 6-*tert*-butyl-diphenylsilylated glucose **66c** (R = TBDPS) could be acylated exclusively at the 2-position employing lipase from *C. cylindracea* (CCL).[164] From the disubstituted glucoses obtained by the enzyme-catalyzed reactions, the protecting functions in the 6-position could be split off chemically or enzymatically, thus making the glucose esters **95** and **96** carrying a single acyl group in the 2- or the 3-position conveniently available (Figure 15).

The monoacylated saccharides used in these studies dissolve in several organic solvents, of which tetrahydrofuran and methylenechloride were found to be particularly suitable for the enzymatic reactions. This was also observed in the lipase-mediated acylation of the methyl glycosides of both D- and L-fucose and -rhamnose, respectively.[184] Using lipase from *Pseudomonas fluorescence* (PFL), both D-carbohydrates were converted to the 2-monobutanoates with high regioselectivity. The naturally occurring L-enantiomers of these 6-deoxysugars, however, were esterified preferably at the 4-hydroxyl groups. These results contrast favorably with chemical derivatizations, since the 4-hydroxyl groups of the 6-deoxy-L-carbohydrates have very little reactivity toward chemical acylating reagents. In addition, methyl-L-fucoside can be converted to the 3-butanoate with lipase from *C. cylindracea*. The introduction of an acyl substituent into the 6-positions of the D-fucoside and the L-rhamnoside does not influence the regioselectivity of the enzymatic acylation.[165]

Finally, it should be mentioned that some attempts were made to differentiate between the hydroxyl groups of fructose by enzymatic methods, but with lipases as well as with subtilisin, only mixtures of 1- and 6-isomers were obtained.[41,150,192] Regioselectively monosubstituted fructoses can, however, be obtained by an enzymatic approach from sucrose (vide infra).

## B. Deprotection of Monosaccharides[133,137]

Initial attempts to apply lipases for the enzymatic removal of acyl groups from glucose pentaacetate resulted in only low levels of selectivity.[193,194] However, at a later time lipase from porcine pancreas (PPL)[168] was found to hydrolyze exclusively the anomeric acetate from peracetylated pyranoses while the esterase from *Rhodosporium toruloides* (RTE)[195] preferably releases the primary hydroxy group (Table 4). On the other hand, if the anomeric center is derivatized as a methyl glycoside, the regioselective enzymatic liberation of the 6-OH group becomes feasible by a number of hydrolytic enzymes.[168,195−199] Thus, from methyl α-D-glucose tetraoctanoate **97a** and the corresponding tetrapentanoate **97b**, lipase from *C. cylindracea* (CCL) removes only the primary ester group in yields of ca. 75%. Similarly, the α-D-galactoside **103** as well as the corresponding mannoside **104b** and the 2-acetamido-2-deoxymannoside **105** were converted to the 6-deprotected pyranosides in 29−50% yield (Table 3), but the 2-acetamido-2-deoxy-glucoside was a very poor substrate. In the latter cases, the regioselectivity was less pronounced and the 4,6-dideoxy derivatives were formed in ca. 20% yield also. In addition to this class of compounds, lipases also accept hexopyranosides carrying several different functionalities (e.g., acetals,[197] enol ethers,[169,200] and, in particular, 1,6-anhydropyranoses as substrates) (Tables 3 and 4). In all cases the reaction conditions are so mild that the acid-sensitive structures of these compounds

**Table 2. Selective Acylation of Secondary Hydroxy Groups in Monosaccharides**

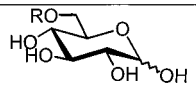
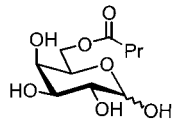
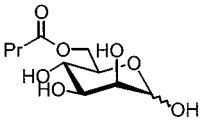
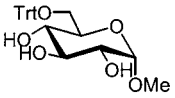
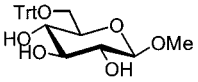
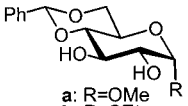
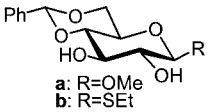
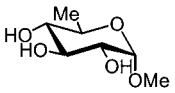
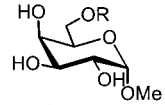
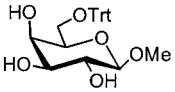
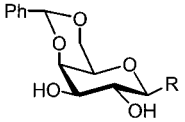
| Compound No. Structure | Enzyme[a] | Solvent | Acyl Donor | Position | Yield (%) | Ref. |
|---|---|---|---|---|---|---|
| **66**  **a**: R=butyryl **b**: R=trityl **c**: R=TBDPS | ANL | THF | $PrCO_2CH_2CCl_3$ | 3 (**66a**) | | [164] |
| | CVL | THF | $PrCO_2CH_2CCl_3$ | 3 (**66a**) | 80 | [164] |
| | PPL | THF | $PrCO_2CH_2CCl_3$ | 2 (**66a**) | 51 | [164] |
| | CVL | THF | $PrCO_2CH_2CCl_3$ | 3 (**66b**) | 88 | [164] |
| | PFL | $MeCO_2CH{=}CH_2$ | $MeCO_2CH{=}CH_2$ | 2 (**66b**) | | [175] |
| | CCL | $CH_2Cl_2$ | $PrCO_2CH_2CCl_3$ | 2 (**66c**) | 45 | [164] |
| **67**  | CVL | THF | $PrCO_2CH_2CCl_3$ | 2<br>3 | 20<br>31 | [164] |
| **68**  | CVL | THF | $PrCO_2CH_2CCl_3$ | 2<br>3 | 13<br>52 | [164] |
| **69**  | lipase from *Mucor miehei* | $MeCO_2CH{=}CH_2$ | $MeCO_2CH{=}CH_2$ | 2 | | [175] |
| **70**  | PFL | $MeCO_2CH{=}CH_2$ | $MeCO_2CH{=}CH_2$ | 3 | | [175] |
| | lipase from *Mucor miehei* | $MeCO_2CH{=}CH_2$ | $MeCO_2CH{=}CH_2$ | 2 | | [175] |
| **71**  **a**: R=OMe **b**: R=SEt **c**: R=OPh | PSL | $RCO_2CH_2CF_3$/THF<br>$RCO_2CH{=}CH_2$/THF | $RCO_2CH_2CF_3$<br>$RCO_2CH{=}CH_2$ | 2 (**71a**)<br>2 (**71a**) | | [176] |
| | PSL | $MeCO_2CH{=}CH_2$ | $MeCO_2CH{=}CH_2$ | 2 (**71a**) | 98 | [177] |
| | PFL | $MeCO_2CH{=}CH_2$ | $MeCO_2CH{=}CH_2$ | 2 (**71a**) | 94 | [178,179] |
| | PFL | *t*$BuCO_2CH{=}CH_2$/THF | *t*$BuCO_2CH{=}CH_2$ | 2 (**71b**)<br>2 (**71c**) | 73<br>76 | [180] |
| **72**  **a**: R=OMe **b**: R=SEt | PSL | $RCO_2CH_2CF_3$/THF<br>$RCO_2CH{=}CH_2$/THF | $RCO_2CH_2CF_3$<br>$RCO_2CH{=}CH_2$ | 3 (**72a**)<br>3 (**72a**) | | [176] |
| | PSL | $MeCO_2CH{=}CH_2$ | $MeCO_2CH{=}CH_2$ | 3 (**72a**) | 86 | [177] |
| | PFL | $MeCO_2CH{=}CH_2$ | $MeCO_2CH{=}CH_2$ | 3 (**72a**) | 86 | [178,179] |
| | PFL | $MeCO_2CH{=}CH_2$ | $MeCO_2CH{=}CH_2$ | 3 (**72b**) | 86 | [180,181] |
| **73**  | PPL | THF/pyridine (4:1) | $PrCO_2CH_2CF_3$ | 2 | 93 | [182] |
| **74**  **a**: R=butyryl **b**: R=trityl **c**: R=benzyl | PPL | THF/pyridine (4:1) | $PrCO_2CH_2CF_3$ | 2 (**74a**) | 84 | [165,182] |
| | PFL | THF/pyridine (4:1) | $PrCO_2CH_2CF_3$ | 2 (**74a**) | 81 | [165] |
| | CCL | $CH_2Cl_2$/pyridine (4:1) | $PrCO_2CH_2CF_3$ | 2 (**74a**) | 80 | [165] |
| | PFL | $MeCO_2CH{=}CH_2$ | $MeCO_2CH{=}CH_2$ | 2 (**74b**) | | [175] |
| | PFL | $MeCO_2CH{=}CH_2$ | $MeCO_2CH{=}CH_2$ | 2 (**74c**) | | [175] |
| **75**  | PFL | $MeCO_2CH{=}CH_2$ | $MeCO_2CH{=}CH_2$ | 3 | | [175] |
| **76**  **a**: R=OAll **b**: R=SEt | PSL | $MeCO_2CH{=}CH_2$ | $MeCO_2CH{=}CH_2$ | 3 (**76a**) | 91 | [177] |
| | PFL | $MeCO_2CH{=}CH_2$/THF | $MeCO_2CH{=}CH_2$ | 3 (**76b**) | 10 | [180] |

**Table 2 (Continued)**

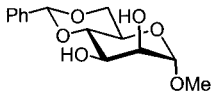
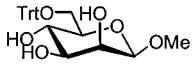
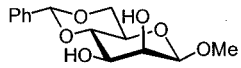
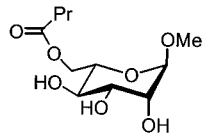
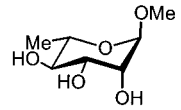
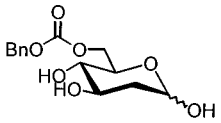
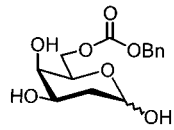
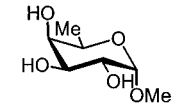
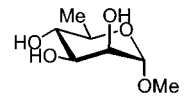
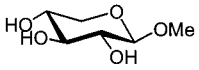
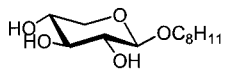
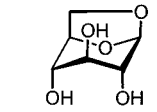
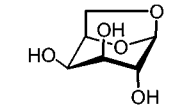
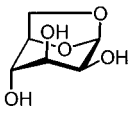
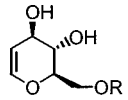
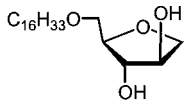
| Compound No. | Structure | Enzyme[a] | Solvent | Acyl Donor | Position | Yield (%) | Ref. |
|---|---|---|---|---|---|---|---|
| **77** |  | PSL | $MeCO_2CH=CH_2$ | $MeCO_2CH=CH_2$ | 2 | 90 | [177] |
| **78** |  | PFL | $MeCO_2CH=CH_2$ | $MeCO_2CH=CH_2$ | 3 | | [175] |
| **79** |  | PSL | $RCO_2CH=CH_2$ | $RCO_2CH=CH_2$ | 3 | 92 | [177] |
| **80** |  | PPL | THF | $PrCO_2CH_2CF_3$ | 4 | 65 | [165] |
| | | PFL | THF | $PrCO_2CH_2CF_3$ | 4 | 68 | [165] |
| **81** |  | PPL | THF/$PrCO_2CH_2CF_3$ (4:1) | $PrCO_2CH_2CF_3$ | 4 | 70 | [183] |
| **82** |  | PSL | dioxane | $RCO_2N=CMe_2$ | 3 | 54-67 | [156] |
| **84** |  | PSL | dioxane | $RCO_2N=CMe_2$ | 3 | 48-56 | [156] |
| **84** |  | PPL | THF/pyridine (4:1) | $PrCO_2CH_2CF_3$ | 2 | 78 | [184] |
| | | PFL | THF/pyridine (4:1) | $PrCO_2CH_2CF_3$ | 2 | 84 | [184] |
| **85** |  | PFL | THF | $PrCO_2CH_2CF_3$ | 2 | 40 | [184] |
| **86** |  | PSL | MeCN | $MeCO_2CH=CH_2$ | 3,4 | 85 | [185] |
| **87** |  | PSL | hexane | $MeCO_2CH=CH_2$ | 2,4<br>3,4 | 70<br>28 | [185] |
| **88** |  | CAL | dioxane | $RCO_2N=CMe_2$ | 4 | 70-72 | [156] |
| | | CAL | dioxane | $MeOCO_2N=CMe_2$ | 4 | 42 | [156] |
| | | PSL | $MeCO_2CH=CH_2$ | $MeCO_2CH=CH_2$ | 4 | | [186,187] |
| | | CAL | $PrCO_2Et$/*t*BuOH | $PrCO_2Et$ | 4; diester | | [188] |
| **89** |  | CAL | $PrCO_2Et$ | $PrCO_2Et$ | 4 | | [188] |

**Table 2 (Continued)**

| Compound No. Structure | Enzyme[a] | Solvent | Acyl Donor | Position | Yield (%) | Ref. |
|---|---|---|---|---|---|---|
| **90**  | PSL | $MeCO_2CH{=}CH_2$ | $MeCO_2CH{=}CH_2$ | 4 | | [187,189] |
| **91**  **a**: R=acetyl **b**: R=benzoyl **c**: R=PhAc | PFL | DME | $RCO_2CH{=}CH_2$ | 3 | 84-93 | [190] |
| **92**  | HLL | benzene | $PrCO_2CH_2CCl_3$ | 2 | 66 | [191] |
| | RJL | benzene | $PrCO_2CH_2CCl_3$ | 3 | 79 | [191] |

[a] Usually many enzymes were screened for activity, only the best results are listed. ANL, *Aspergillus niger* lipase; CAL, *Candida antarctica* lipase; CCL, lipase from *Candida cylindracea* (later renamed *Candida rugosa*; CRL); CVL, *Chromobacterium viscosum* lipase; HLL, *Humicula lanuginosa* lipase; PFL, *Pseudomonas fluorescens* (later renamed *Pseudomonas cepacia*) lipase; PPL, porcine pancreas lipase; PSL, *Pseudomonas cepacia* lipase; RJL, *Rhizopus japonicus* lipase.

remain unaffected. Particularly remarkable is the regioselectivity displayed by lipase from *P. cepacia* (PSL) in the deprotection of the glycal **131**.[169,200] The biocatalyst exclusively attacks the 3-acetate and leaves the primary ester intact. The enzymatic deprotection strategy can also be used to synthesize carbohydrates carrying a single acyl group in selected positions. Thus, 3,6-dibutyryl glucose **93** (prepared by enzymatic acylation of glucose) was converted to the 3-butanoate **95** by lipase-mediated hydrolysis of the 6-ester (Figure 15).[164]

The principles and the enzymes mentioned above which allow the regio- and chemoselective protection and deprotection of the various pyranoses to be carried out were also successfully applied for the enzymatic manipulation of acyl groups in furanoses. Of particular interest in this context is the finding that the five-membered rings can also be handled by the biocatalysts with a pronounced regioselectivity, although furanoses can adopt more flexible conformations with close energy in solution.

The cleavage of the primary acetyl groups from the furanosides **106**−**111** could be carried out in high yields with lipase from *C. cylindracea* (Table 3).[168] For the 2-deoxy-α-D-ribofuranoside and the α- and *β*-xylo compounds the hydrolysis was less selective. From the peracetylated furanoses **125** and **126**, the anomeric acyl group was removed with complete selectivity by means of lipase from *A. niger* (Table 4).

1,6-Anhydropyranoses serve as convenient starting materials for various synthetic purposes in carbohydrate chemistry. Therefore, the directed manipulation of their hydroxyl groups is of particular interest. Each of the three OH groups in 1,6-anhydroglucopyranose can be liberated selectively, making use of enzymatic reactions (Figure 16, Table 4).[208−210,212] Thus, from the triacetate **127a**, the 4-protecting group was split off using lipase from porcine pancreas (PPL)[209] or pig liver esterase (PLE).[208,209] The acetate

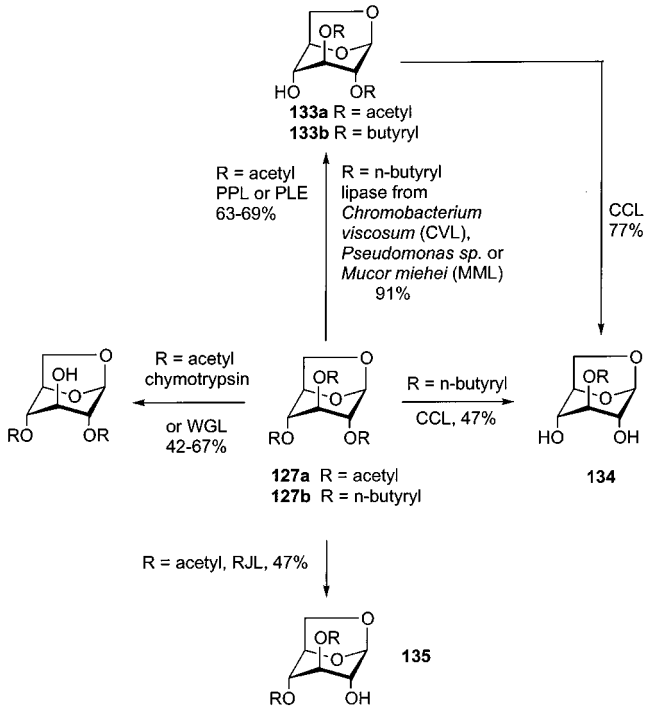



**Figure 16.** Selective enzymatic removal of protecting groups from 1,6-anhydropyranoses.

in the 3-position could be attacked preferentially using chymotrypsin[209] or lipase from wheat germ (WGL),[208] and the 3,4-diacetate **135** was obtained by hydrolysis with lipase from *Rhizopus javanicus* (RJL).[208] In each case, however, other derivatives were formed as undesired byproducts. High yields could be obtained from the tri-*n*-butanoate **127b**. It was converted to the 2,3-dibutanoate **133b** in 91% yield by means of several lipases, but the enzyme from *C. cylindracea* (CCL) removed two acyl groups successively to yield the monobutanoate **134**. Similarly, the analogous 3-azido-1,6-anhydropyranose **128** is regioselectively deacylated at O-2 and O-4 by means of lipase OF from *C. cylindracea* and alcalase, respectively.[211] Of particular importance is the ster-

**Table 3. Selective Deacylation of Primary Hydroxy Groups in Monosaccharides**

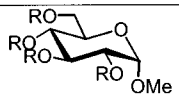
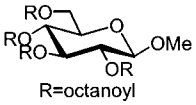
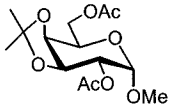
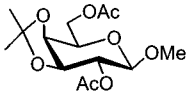
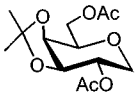
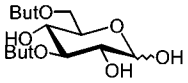
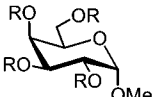
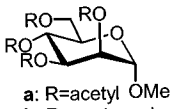
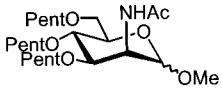
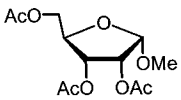
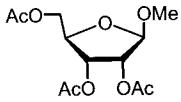
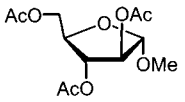
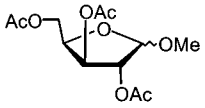
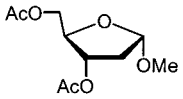
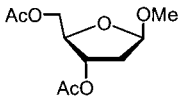
| Compound No. | Structure | Enzyme[a] | Solvent | Position | Yield (%) | Ref. |
|---|---|---|---|---|---|---|
| **97** |  **a**: R=octanoyl **b**: R=pentanoyl **c**: R=acetyl | CCL | 0.1 M phosphate buffer | 6 | 78 (**97a**) | [168] |
| | | CCL | 0.1 M phosphate buffer | 6 | 75 (**97b**) | [168] |
| | | CCL | 0.1 M phosphate buffer | 6 | 90 (**97b**) | [196] |
| | | CCL | 0.1 M phosphate buffer, $Bu_2O$ (10 %) | 6 | (**97c**) | [197] |
| | | PEG-modified CCL | $Cl_3CCH_3$ | 6<br>4,6 | 27 (**97c**)<br>48 (**97c**) | [198] |
| | | CRL | 0.1 M phosphate buffer | 6 | 91 (**97c**) | [199] |
| | | RTE | citrate buffer | 6 | 77 (**97c**) | [195] |
| **98** |  R=octanoyl | CCL | 0.1 M phosphate buffer | 6 | 77 | [168] |
| **99** |  | PPL | 0.1 M phosphate buffer, acetone 10:1 | 6 | 90 | [166] |
| **100** |  | PPL | 0.1 M phosphate buffer, acetone 10:1 | 6 | 82 | [166] |
| **101** |  | PPL | 0.1 M phosphate buffer, acetone 10:1 | 6 | 75 | [166] |
| **102** |  | CCL | 0.1 M Tris•HCl | 6 | 85 | [164] |
| **103** |  **a**: R=acetyl **b**: R=pentanoyl | RTE | citrate buffer | 6 | 85 (**103a**) | [195] |
| | | CCL | 0.1 M phosphate buffer | 6 | 29 (**103b**) | [168] |
| **104** |  **a**: R=acetyl **b**: R=pentanoyl | CRL | 0.1 M phosphate buffer | 6 | 94 (**104a**) | [199] |
| | | RTE | citrate buffer | 6 | 70 (**104a**) | [195] |
| | | CCL | 0.1 M phosphate buffer | 6 | 33 (**104b**) | [168] |
| **105** |  | CCL | 0.1 M phosphate buffer | 6 | 50 | [168] |
| **106** |  | CCL | 0.1 M phosphate buffer, 10 % DMF | 6 | 85 | [168] |
| **107** |  | CCL | 0.1 M phosphate buffer, 10 % DMF | 5 | 96 | [168] |

**Table 3 (Continued)**

| Compound No. | Structure | Enzyme[a] | Solvent | Position | Yield (%) | Ref. |
|---|---|---|---|---|---|---|
| **108** |  | CCL | 0.1 M phosphate buffer, 10 % DMF | 5 | 98 | [168] |
| **109** |  | CCL | 0.1 M phosphate buffer, 10 % DMF | 5<br>3 | 50<br>30 | [168] |
| **110** |  | CCL | 0.1 M phosphate buffer, 10 % DMF | 5<br>3 | 40<br>50 | [168] |
| **111** |  | CCL | 0.1 M phosphate buffer, 10 % DMF | 5 | 63 | [168] |

[a] Usually many enzymes were screened for activity, only the best results are listed. ANL, *Aspergillus niger* lipase; CCL, lipase from *Candida cylindracea* (later renamed *Candida rugosa*; CRL); PPL, porcine pancreas lipase; RTE, *Rhodosporium toruloides* esterase.

eochemistry at C-4 of the bicyclic substrates. If the alcohol at this position is equatorial, as, for instance, in the corresponding 1,6-anhydrogalactopyranose **129** and the analogous lactone **130**, several enzymes act only in a random fashion or not at all.[210] However, the acyl group in the 2-position seems to be preferred (Table 4). The results obtained from these studies indicate that the reactivity of acyl protecting groups in 1,6-anhydropyranoses toward hydrolysis by lipases decreases in the order C-$4_{ax}$ > C-$2_{ax}$ > C-$3_{ax}$ ≫ C-$4_{eq}$.

The above-mentioned investigations unraveled that the lipase-mediated hydrolysis proceeds at higher reaction rate and in many cases with better selectivity if butanoates or pentanoates are employed as substrates instead of acetates. However, by no means is the use of enzymatic deacylations restricted to simple alkanoates. An illustrative and impressive example is found in the hydrolysis of generally base-stable carbohydrate pivaloylates using an esterase from rabbit serum (ERS).[214−217] For instance, the biocatalyst selectively splits off the 6-pivaloyl group from α-methyl 3,4,6-tripivaloyl-2-acetamido-2-deoxy-glucoside. On prolonged incubation, the complete removal of pivaloylates from carbohydrates is possible also. Of particular significance is that the enzyme does not have to be purified but that crude serum preparations are sufficient for the preparative purposes. A further enzyme which allows the chemo- and regioselective unmasking of different carbohydrate derivatives to be carried out is acetyl esterase from the flavedo of oranges, a biocatalyst which hydrolyzes preferably acetic acid esters.[32,218] It can be applied for the synthesis of selectively deacylated pyranoses. Thus, from pentaacetylglucose **112**, the 2,3,4,6-tetraacetate is obtained by means of the regioselective saponification of the 1-acetate. If the hydrolysis is allowed to proceed further, the 6-acetate is cleaved also and the 2,3,4-triacetate becomes available in ca. 40% yield. If tri-*O*-acetyl-glucal **131** is subjected to the enzymatic hydrolysis, at 40% conversion the 6-acetate is the main product.

By introducing acyl groups which are specifically recognized by certain enzymes into carbohydrates, not only the regioselectivity but also the chemoselectivity of the biocatalysts can be exploited. This can, for instance, be achieved by the selective saponification of phenylacetates catalyzed by penicillin G acylase.[30−32] The enzyme liberates the 2-OH group of 1,3,4,6-tetraacetyl-2-phenylacetyl glucose without affecting the acetic acid esters. In this case, moreover, an ester of a secondary hydroxyl function is chemoselectively hydrolyzed in the presence of the chemically more reactive acetates at the 6-position and at the anomeric center. This approach was also adopted for the enzymatic deprotection of the glucal **132**. Thus, its 3-OH group was liberated without cleaving the acetates present.[190]

## C. Di- and Oligosaccharides[137]

For enzymatic protecting group manipulations on di- and oligosaccharides in particular, the use of subtilisin together with dimethylformamide as solvent is advantageous. As already pointed out, the use of DMF is often critical, since its dissolving ability is high enough to solubilize even highly polar polyhydroxy compounds (comparable experiments with pyridine as solvent mainly failed).[141] Very few reports about the successful use of other solvents such as pyridine[219] or *tert*-butyl alcohol[220] have been published.

Subtilisin accepts several disaccharides as substrates and transfers butyric acid from ethyl or trichloroethyl butanoate to the primary 6′-hydroxyl functions of the nonreducing monosaccharide of the β-(1−3)-linked cellobiose **136** and the respective maltobiose (Figure 17).[150,220] For lactose the regioselectivity was less pronounced; however, methyl and

**Table 4. Selective Deacylation of Secondary Hydroxy Groups in Monosaccharides**

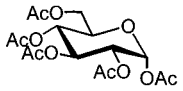
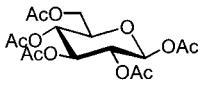
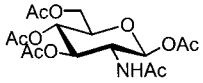
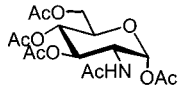
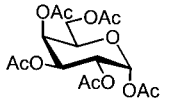
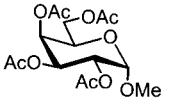
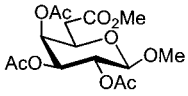
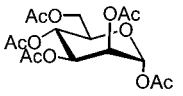
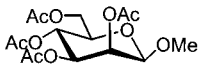
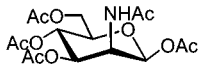
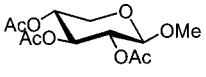
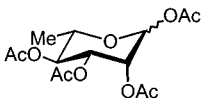
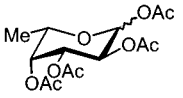
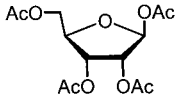
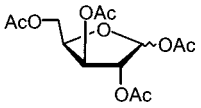
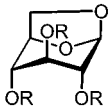
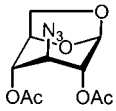
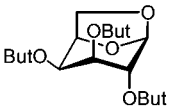
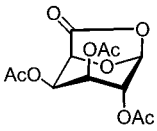
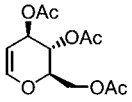
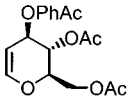
| Compound No. | Structure | Enzyme[a] | Solvent | Position | Yield (%) | Ref. |
|---|---|---|---|---|---|---|
| **112** |  | CCL | 0.1 M phosphate buffer | 4,6 | 73 | [168] |
| | | RTE | citrate buffer | 6 | 54 | [195] |
| | | PPL | 0.05 M phosphate buffer, 10 % DMF | 1 | 70 | [168] |
| | | PFL | phosphate buffer, MeCN (7:3) | 1 | 80 | [201] |
| | | CCL | phosphate buffer, MeCN (7:3) | 4 | 50 | [201] |
| | | CCL | phosphate buffer, MeCN (7:3) | 6 | 75 | [201] |
| **113** |  | PPL | 0.05 M phosphate buffer, 10 % DMF | 1 | | [168] |
| | | CAL | butanone | 1 | 95 | [202] |
| **114** |  | PPL | 0.05 M phosphate buffer, 10 % DMF | 1 | 96 | [168] |
| **115** |  | ANL | phosphate buffer, MeCN (10:1) | 1,4 | 41 | [203] |
| | | RTE | citrate buffer | 6 | 80 | [204] |
| **116** |  | PPL | 0.05 M phosphate buffer, 10 % DMF | 1 | 75 | [168] |
| | | RTE | citrate buffer | 6 | 67 | [195] |
| **117** |  | ANL | 0.1 M phosphate buffer, 10 % acetone | 2 | 58 | [205] |
| **118** |  | *hog kidney* acylase | phosphate buffer, DMF (10:1) | 2 | 93 | [206] |
| | | *Aspergillus niger* pectinase | phosphate buffer, DMF (10:1) | 3 | 27 | [206] |
| | | ANL | phosphate buffer, DMF (10:1) | 4 | 11 | [206] |
| **119** |  | PPL | 0.05 M phosphate buffer, 10 % DMF | 1 | 95 | [168] |
| | | RTE | citrate buffer | 6 | 88 | [195] |
| **120** |  | ANL | 0.1 M phosphate buffer, 10 % acetone | 3 | 61 | [205] |
| **121** |  | PPL | 0.05 M phosphate buffer, 10 % DMF | 1 | 88 | [168] |
| **122** |  | PSL | *t*amyl-OH | 4 | 84 | [207] |
| | | PEG-modified CCL | $Cl_3CCH_3$ | 4 | 82 | [198] |
| **123** |  | PPL | 0.05 M phosphate buffer, 10 % DMF | 1 | 54 | [168] |
| **124** |  | PPL | 0.05 M phosphate buffer, 10 % DMF | 1 | 71 | [168] |
| **125** |  | ANL | 0.1 M phosphate buffer, 10 % DMF | 1 | 63 | [168] |

**Table 4 (Continued)**

| Compound No. | Structure | Enzyme[a] | Solvent | Position | Yield (%) | Ref. |
|---|---|---|---|---|---|---|
| **126** |  | ANL | 0.1 M phosphate buffer, 10 % DMF | 1 | 50 | [168] |
| **127** |  **a**: R=acetyl **b**: R=butyryl | RJL | 0.1 M phosphate buffer | 2<br>4 | 47 (**127a**)<br>15 (**127a**) | [208] |
| | | WGL | 0.1 M phosphate buffer | 3 | 67 (**127a**) | [208] |
| | | PLE | 0.1 M phosphate buffer | 4 | 69 (**127a**) | [208] |
| | | PPL | 0.05 M citrate-phosphate buffer | 4 | 42 (**127a**) | [209] |
| | | CVL | 0.1 M phosphate buffer | 4 | 91 (**127b**) | [210] |
| | | CCL | 0.1 M phosphate buffer | 2,4 | 77 (**127b**) | [210] |
| **128** |  | CCL | 0.1 M phosphate buffer | 4 | 85-90 | [211] |
| | | alcalase | 0.1 M phosphate buffer | 2 | 82 | [211] |
| **129** |  | CCL | 0.1 M phosphate buffer | 2 | | [210] |
| | | CCL | 0.1 M phosphate buffer | 2 | 90 | [212] |
| | | PPL | 0.1 M phosphate buffer | 2<br>4<br>2,4 | 16<br>19<br>65 | [212] |
| **130** |  | WGL | phosphate buffer, DMF (10:1) | 2 | 60 | [206] |
| **131** |  | PSL | 0.25 M phosphate buffer | 3 | 90 | [169] |
| | | acetyl esterase from the *flavedo* of oranges | 0.15 M NaCl buffer | 3,4<br>3,4,6 | 24<br>22 | [74,213] |
| **132** |  | PGA | 0.1 M phosphate buffer | 3 | 80-85 | [190] |

[a] Usually many enzymes were screened for activity, only the best results are listed. ANL, *Aspergillus niger* lipase; CAL, *Candida antarctica* lipase; CCL, lipase from *Candida cylindracea* (later renamed *Candida rugosa*; CRL); PGA, penicillin-G-acylase; PLE, porcine liver esterase; PPL, porcine pancreas lipase; PSL, *Pseudomonas cepacia* lipase; RJL, *Rhizopus japonicus* lipase; RTE, *Rhodosporium toruloides* esterase; WGL, wheat germ lipase.

benzyl $\beta$-D-lactoside **137** were converted to the 6′-butanoates in 71−73% yield.[221] Rutinose, in which the primary hydroxy group of the glucose moiety is blocked (see also **149**, Figure 19), is selectively substituted in the 3-position.[222] In addition, higher maltooligomers could be acylated in the 6-position of the terminal nonreducing carbohydrate also. For instance, 6″-*O*-butyrylmaltotriose was isolated in 29% yield, but the corresponding tetra-, penta-, hexa-, and heptamer were substrates for the biocatalyst also. These enzymatic esterifications open a route to discriminate between the primary hydroxyl groups in di- and oligosaccharides in a convenient and straightforward way. Classical chemical one-step methods of comparable selectivity are not available for this purpose,[139,140] and usually multistep sequences have to be carried out if the selective protection of a specific primary hydroxyl group in a di- or oligosaccharide is desired.

Due to its great commercial importance as a renewable resource, sucrose **138** has been subjected to several enzymatical hydroxyl group manipulations. This nonreducing disaccharide turned out to be a substrate for subtilisin also.[150] In contrast to chemical acylations in which the most reactive OH groups are found in the 6- and 6′-positions, the enzyme selectively transfers various acyl functions to the 1′-alcohol (Figure 17).[150,192,223] This acylation was usually carried out in DMF as a solvent, but the use anhydrous pyridine gave similar results.[219] The monoacylated disaccharides **139** thereby obtained could then be further transformed enzymatically. On one hand, with the lipase from *C. viscosum* (CVL) the free primary 6-OH group was acylated in 31% yield. On the other hand, the 1′-esters **139** are substrates for yeast α-glucosidase which hydrolyzes the glycosidic bond and thus makes the 1-*O*-acylfructoses **140**, potentially useful as chiral synthons,

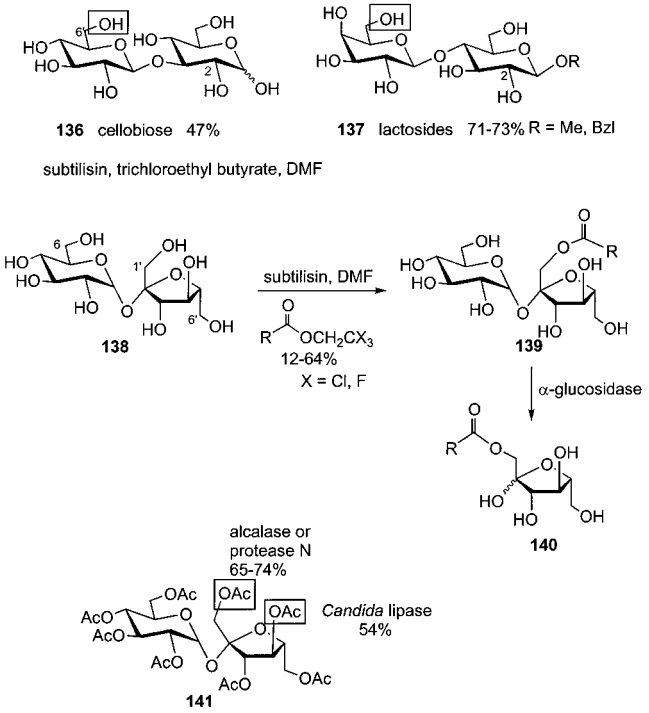



**Figure 17.** Selective enzymatic protection and deprotection of disaccharides.

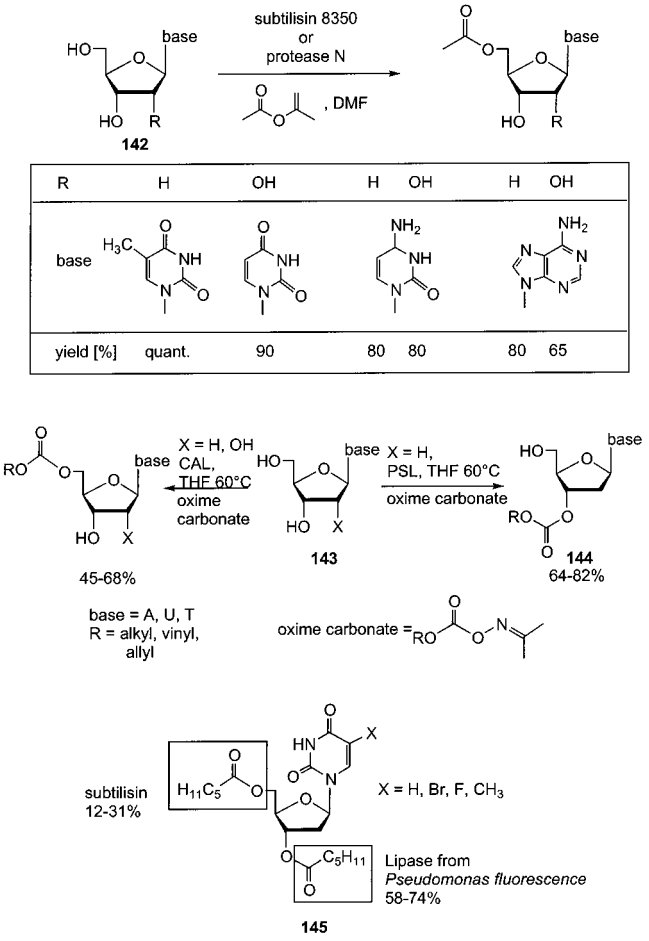



**Figure 18.** Selective enzymatic protection and deprotection of the carbohydrate parts of nucleosides.

available.[192] Alternatively, the 6′-OH group in sucrose **138** can be selectively acylated if the carbohydrate

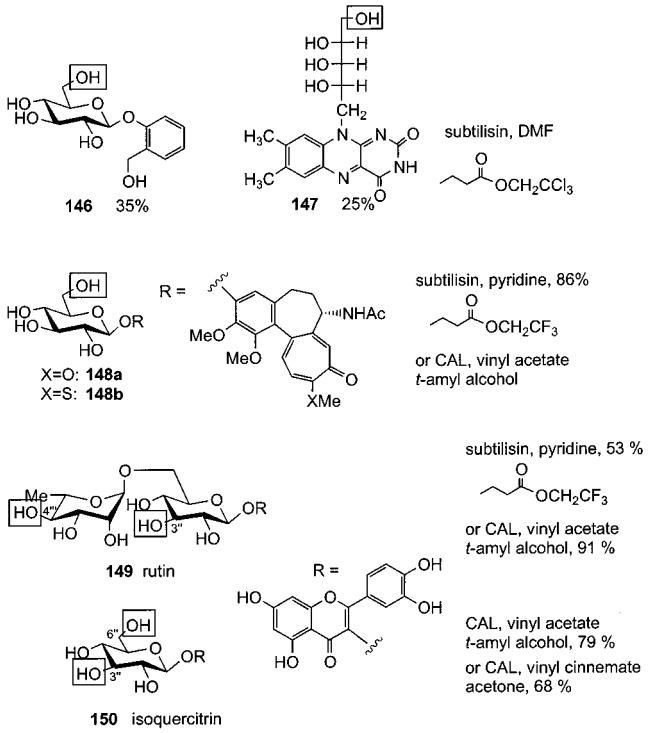



**Figure 19.** Selective enzymatic acylation of aglycon glycosides.

is converted to the 2,1′:4,6-bisacetal prior to the treatment with a lipase (Novozym 435).[224]

In the direction of hydrolysis several enzymes were investigated.[225−229] Depending on the biocatalyst used, acetyl groups from different positions of octaacetyl sucrose **141** could be removed selectively in useful yields. For instance, alcalase and protease N preferably attack the acetate on O-1′,[226,230] the lipase from *C. cylindracea* preferably liberates the OH group on C-4′ of the furanoid ring,[225,230] and wheat germ lipase preferentially liberates the 1′-, 4′-, and 6′-OH groups (Figure 17).[223,231]

The deacylation of the octaacetates of cellobiose, lactose, maltose, and melibiose with *A. niger* lipase leads to the formation of the respective carbohydrate heptaacetates with a free anomeric OH group at C-1 in high yield.[230,232] Upon prolonged reaction times, the acetates at C-1 and C-2 are hydrolyzed from cellobiose and lactose octaacetate in 51% and 42% yields, respectively.

## D. Nucleosides[135,233]

The directed protection of nucleoside functional groups is a fundamental problem in nucleoside and nucleotide chemistry. Although several chemical methods are available for the regioselective acylation of the nucleoside carbohydrates, enzymatic methods offer significant advantages with respect to yield, regioselectivity, and the number of synthetic steps which have to be carried out.

Earlier studies focused on the use of the dihydrocinnamoyl group as an enzyme-labile nucleoside protecting function which can be removed through use of α-chymotrypsin.[234,235] Although the enzyme shows an interesting tendency to attack preferably the 5′-position, this technique was not further ex-

ploited. Highly regiodiscriminating biocatalyzed acyl transfer reactions to the carbohydrate parts of various nucleosides could be carried out employing the protease subtilisin together with dimethylformamide as solvent. In particular, a mutant of this enzyme, obtained via site-specific mutations, appears to display advantageous properties. It transfers the acetyl group from isopropenyl acetate to the primary hydroxyl functions of various purine and pyrimidine nucleosides and 2′-deoxynucleosides **142** in high yields (Figure 18).[236] Commercially available subtilisin (protease N from Amano) provided the same compounds with identical yields and selectivities; however, five times more enzyme is required for this purpose. In addition, in the transfer of butyric acid from trichloroethyl butanoate to adenosine and uridine, carried out earlier,[150] this biocatalyst showed inferior properties with respect to regioselectivity and yields.

The selective introduction of protecting groups into the hydroxyl functions of different nucleosides can be achieved by means of lipases also. Thus, unprotected pyrimidine and purine 2′-deoxynucleosides **143** (X = H) are selectively converted to the 3′-*O*-acylated derivatives **144** in 64−82% yield making use of lipase from *P. cepacia* (PSL) and employing oxime carbonates as acyl donors (Figure 18).[237−239] Similarly, by applying oxime esters or acid anhydrides, different ester functions can be selectively introduced into the 3′-position of nucleosides by using the lipases from *C. cylindracea* (CCL), porcine pancreas (PPL), or *P. cepacia* (PSL).[240−244] If lipase from *Candida antarctica* (CAL) is used, however, the esters and carbonates are predominantly generated at the primary 5′-OH group of (deoxy)nucleosides.[238,239,241,242,244−247] Furthermore, in the case of ribonucleosides, complete regioselectivity can be achieved by using the same methodology.[241] The regioselectivity of the CAL-catalyzed alkoxycarbonylation is profoundly influenced by the structure of the starting oxime carbonate.[248] In the alkoxycarbonylation of thymidine, use of the phenyl derivative leads to almost exclusive formation of the 5′-carbonate while the corresponding allyl carbonate is introduced without any regioselectivity.

An investigation of the enzyme-catalyzed acylation of α-, xylo-, anhydro-, and arabino-nucleosides showed that in these cases the primary 5′-hydroxy group can be selectively acylated using lipase from *Candida antarctica* (CAL).[249−251] A selective derivatization of the 3′-OH group, however, was unsuccessful.

Acylations of nucleosides with acid anhydrides in the presence of lipase from *P. fluorescence* (PFL) with DMF or DMSO as solvent first proceeded with unsatisfying regioselectivity.[252] However, this lipase together with subtilisin can be applied to effect highly specific deacylations of various pyrimidine nucleosides **145** (Figure 18).[253] Thus, lipase from *P. fluorescence* (PFL) preferably attacks the hexanoyl group on the secondary hydroxyl function of the N-glycosides, giving rise to the 5-esters in good yields. On the other hand, subtilisin makes the 3-esters available with moderate results. It should be noted, however, that in both cases considerable to large amounts (6−71%) of the completely deprotected nucleosides were formed also. Subtilisin in phosphate buffer also selectively hydrolyzes the 5′-acetate of purine- and pyrimidine-triacetylated esters to give the corresponding 2′,3′-diacetylribonucleosides in 40−92% yield.[254] A similar preference was observed for the lipase from porcine pancreas but with poorer selectivity and a slower reaction rate. This enzyme, however, deacetylated the deoxynucleoside 3′,5′-di-*O*-acetylthymidine at the 5′-position in almost quantitative yield.[255] In contrast, if lipase from *C. cylindracea* (CCL) was used in the catalysis, the 3′-ester of this diacetate was preferentially hydrolyzed.[255]

Using acetyl esterase of the *flavedo* of oranges, bisacylated purine deoxynucleotides can be selectively deprotected at the 3′-hydroxy group in 31−40% yield.[74] Interestingly, by introducing a phenylacetyl group for amino protection in the purine moiety, the regioselectivity of the acetyl removal is reversed. Now the primary acetate is hydrolyzed by acetyl esterase in 22−52% yield.

Additionally, the complete hydrolysis of an anomeric mixture of peracetylated 2′-deoxynucleosides by wheat germ lipase or porcine liver esterase has been used to synthesize the pure β-anomer of, e.g., thymidine as the only completely deprotected product.[256] The alcoholysis of peractylated uridines catalyzed by *Candida antarctica* lipase leads to the formation of the completely deprotected nucleoside.[257] Although this reaction can be stopped after removal of the first acetyl group, no regioselectivity was observed for the formation of di-*O*-acetyluridine.

## E. Further Aglycon Glycosides

In addition to nucleosides, several other naturally occurring carbohydrate derivatives can be selectively protected/deprotected by means of enzymatic techniques. For instance, salicin **146**, a wood component that contains a primary hydroxyl group located in a glucose moiety and a second one in a benzylic position, was butyrylated exclusively at the 6-OH of the monosaccharide in 35% yield by applying subtilisin and trichloroethyl butanoate in DMF (Figure 19).[150] Under the same conditions, in riboflavin (vitamin $B_2$) **147** only the primary alcohol was esterified in 25% yield,[150] and colchicoside **148a** as well as a thio analogue **148b** were converted to the 6′-butanoates by treatment with trichloroethyl butanoate in pyridine in the presence of subtilisin.[258] The corresponding 6′-acetates of **148a,b** were obtained by treatment with vinyl acetate in the presence of *Candida antarctica* lipase as the biocatalyst (Figure 19).[162] Similarly, the carbohydrate parts of flavonoid disaccharides were regioselectively functionalized. Thus, for instance, in the disaccharide rutin **149** and the related hesperidin, only the 3″-OH group of the glucose moiety was esterified upon treatment with trifluoroethyl butanoate and subtilisin in 53% yield (Figure 19).[222] In the presence of lipase from *C. antarctica*, however, both the 3″- and the 4‴-positions were acetylated.[162] If only the glucose moiety is present in the molecule, as in the related isoquercitrin **150**, the regioselectivity in the subtilisin-catalyzed reaction was less pronounced.[259]

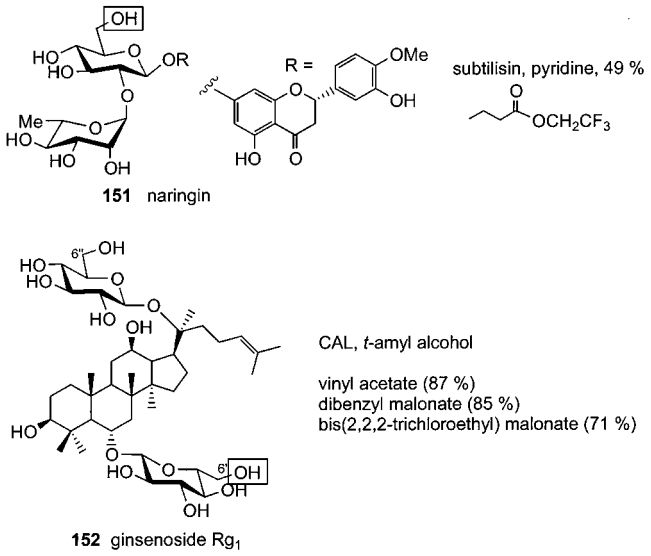



**Figure 20.** Selective enzymatic acylation of aglycon glycosides.

However, in the presence of lipase from *C. antarctica*, the 3″,6″-bisacylated product is formed if vinyl acetate is used as the acyl donor.[162] Interestingly, by using vinyl cinnamate as an acyl donor, this biocatalyst only acylates the primary 6″-hydroxy group.[260] Naringine **151** was converted into the 6-glucosyl ester in the presence of subtilisin (Figure 20). In all cases the rhamnose and the phenolic hydroxyls remained unattacked (for the protection of phenolic hydroxy groups in flavonoids see section V.H).

The steroidal glucoside ginsensoside $Rg_1$ **152** can be selectively monoacylated in high yields at the 6′-position using *C. antarctica* lipase as the biocatalyst.[61,262] In this case, similar results were obtained with different acyl donors such as vinyl acetate, dibenzyl malonate, and bis(trichloroethyl) malonate (Figure 20).

Two impressive examples for selective enzymatic deacylations of complex substrates consist of the removal of all acetates from the peracetylated $\beta$-D-glucopyranosyl ester **153** of abscisinic acid[263] and the gibberellinic acid derivative **154**,[264] containing one glucose tetraacetate glycosidically bound and a second one attached as an ester (Figure 21). In both cases the removal of the acetyl groups by chemical methods, in particular, was complicated by an undesired cleavage of the ester linkages to the glucoses. However, the four acetyl groups present in **153** could be hydrolyzed chemoselectively by means of helicase, an enzyme occurring in the seeds of *Helianthus annus*, whereby the unprotected glucose ester was formed in 82% yield without destroying the ester bond between abscisinic acid and glucose. Similarly, the biocatalyst removed all acetates from **154**. In this case the yield reached only 8%; it should, however, be kept in mind that 10 acetic acid esters had to be cleaved in the enzymatic process and that the aglycon is rather complex.

In conclusion, the various enzyme-mediated protecting group manipulations carried out on numerous carbohydrate derivatives indicate that biocatalysts can advantageously be used in the protecting group chemistry of carbohydrates. In particular, subtilisin

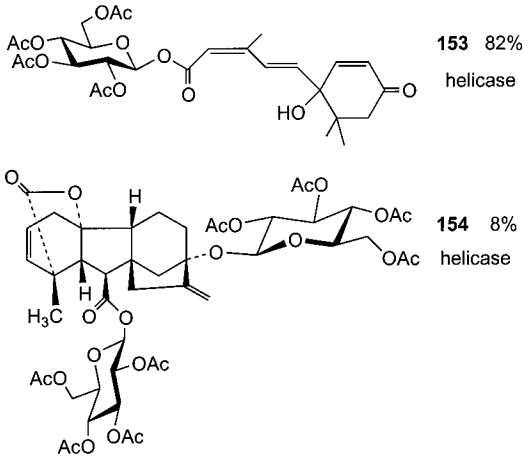



**Figure 21.** Enzymatic deprotection of complex glucosyl esters.

and several lipases from different sources (from porcine pancreas, *C. cylindracea*, *A. niger*, *C. viscosum*, *Mucor javanicus*, *P. fluorescence*, and wheat germ) allow the chemo- and regioselective acylation and deprotection of various saccharides, whose structures differ widely, to be carried out. A general principle that emerges from these studies is that the enzymes exhibit a predominant preference toward primary hydroxyl groups. If these functionalities are not present or protected, the biocatalysts are capable of selectively manipulating secondary hydroxyl groups or esters thereof. In the introduction and removal of acyl groups, the regioselectivity displayed by the enzymes often parallels the findings recorded for classical chemical transformations, although it is significantly higher in many cases. Furthermore, in several cases regioselectivities were observed in the biocatalyzed processes which cannot or can only hardly be achieved by means of chemical methods. Finally, it should be realized that subtilisin and the lipases are capable of introducing specific acyl groups into the carbohydrates which can afterward be removed selectively by different enzymatic or chemical methods.

## F. Polyhydroxylated Alkaloids

The plant alkaloid castanospermine **155** and the related piperidine alkaloid 1-deoxynojirimicin **160**, like several other polyhydroxylated octahydroindolizidines, piperidines, and pyrrolidines, are potent glycosidase inhibitors. These nitrogen bases are of considerable interest for the study of biosynthetic processes, and in addition, castanospermine and some of its derivatives may be of clinical value as antineoplastic agents and as drugs in the treatment of AIDS.

In light of the analogy between the structures of these alkaloids and glucose, some of the abovementioned enzymatic methods for the selective functionalization of carbohydrates were applied to prepare several acyl derivatives of **155** and **160**. Thus, subtilisin transfers the acyl moieties from several activated esters to the 1-OH group of the bicyclic base in moderate to high yields (Figure 22).[265,266] Again, pyridine had to be used as solvent for the polyhydroxy

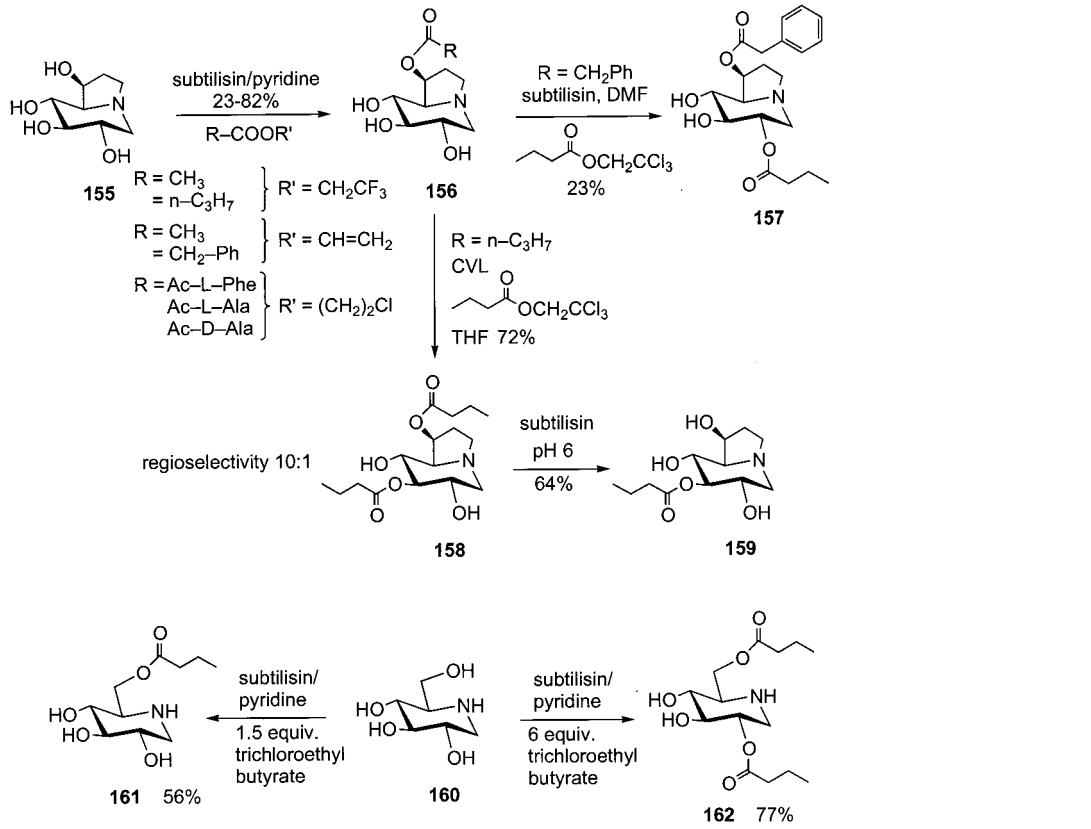



**Figure 22.** Selective enzymatic protection of polyhydroxylated alkaloids.

compound. The monoesters **156** obtained by this technique, like the monoesters of hexoses, could subsequently be dissolved in THF and were further acylated by means of different enzymes, e.g., to the 6-butanoate **157** and the 1,7-dibutanoate **158**. Finally, the 1-ester was removed from **158** by subtilisin in aqueous solution to deliver the 7-butanoate **159** in 64% yield.

In contrast to castanospermine, 1-deoxynojirimicine **160** contains a primary hydroxyl group as well as a much more nucleophilic amino function. If a small excess of trifluoroethyl butanoate is employed, subtilisin converts this alkaloid preferably into the 6-monoester **161** (Figure 22).[266] However, with 6 equiv of the acylating agent, the 2,6-diester **162** is formed in 77% yield. **162** may be subsequently deacylated regioselectively at the 6-position by means of several different enzymes.

It should be noted that under the conditions of the enzymatic acylation, the amino group is not derivatized, an observation which has also been made in related cases,[266,267] e.g., N-terminally deprotected serine-peptides.

## G. Steroids

Enzymatic acyl transfer reactions also are practical processes for the acylation of hydroxyl groups in steroids. The lipase from *C. viscosum* (CVL), for instance, selectively transfers butyric acid from trifluoroethyl butanoate to equatorial ($\beta$) C-3-alcoholic functions present in a variety of sterols, e.g., **163** and the respective 5,6-didehydro compound (Figure 23).[268] Axially oriented alcohols at C-3 and secondary alcohols at C-17 or in the sterol side chains are not

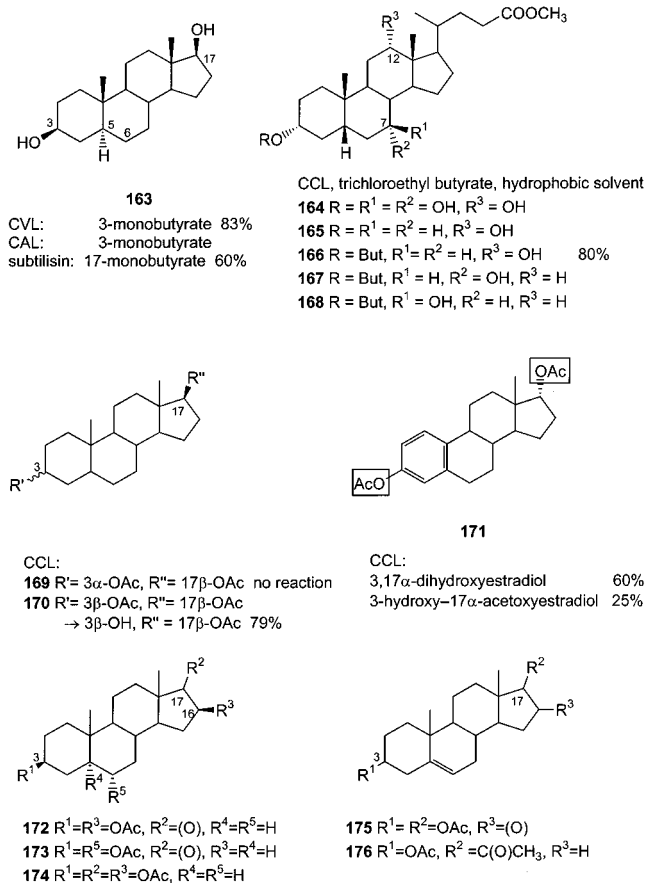



**Figure 23.** Selective enzymatic protection of steroids.

derivatized. In addition to the equatorial alcohols, the compounds being accepted as substrates by the lipase

must have the A/B-ring fusion in the trans configuration. In the B-ring a double bond is tolerated; in the A-ring, however, it is not. Similarly, lipase from *Candida antarctica* acylates the 3-hydroxy group in steroids such as **163** and its 5,6-didehydro derivative.[269] Interestingly, acylation in this position is preferred regardless of the orientation of the hydroxy group. For instance, treatment of **164** with vinyl acetate in the presence of *C. antarctica* lipase leads to the formation of the corresponding 3-acetylated derivative in 82% yield. In contrast, subtilisin does not recognize the hydroxyl group at C-3 of the steroid nucleus but rather transfers the acyl moiety to alcoholic groups in the 17-position or in the side chains (Figure 23). Changes in the A- or B-ring do not dramatically influence the selective mode of action of this biocatalyst. This behavior equals the one determined for the lipase of *P. cepacia*, which was recently used for the regio- and stereoselective acylation of steroids.[270] Thus, using these enzymes, the completely regioselective protection of either alcoholic group in several steroid diols is possible. This feature opened a route to a new chemoenzymatical process for the oxidation of selected positions of the steroid framework via an enzymatic protection/oxidation/deprotection sequence. Chemoenzymatic approaches of this type are expected to provide attractive alternatives to the currently utilized enzymatic oxidation of steroids by hydroxysteroid dehydrogenases.

A further biocatalyst comes into play when bile acids serve as starting materials, e.g., deoxycholic acid methyl ester **165**.[271] The cis-configuration of the A/B-ring fusion prevents the application of lipase from *C. viscosum* (CVL), and the aliphatic chain hinders the esterification of the C-12α hydroxyl group by subtilisin. The lipase from *C. cylindracea* (CCL) has proven to be the most suitable enzyme for the enzymatic acylation of bile acids. In hydrophobic solvents, i.e., hexane, toluene, butyl ether, benzene, etc. (except acetone), and employing trichloroethyl butanoate as acyl donor, the 3α-*O*-butanoyldeoxycholic acid methyl ester **166** is formed in 80% yield without any byproducts, suggesting that the enzyme is ineffective toward 12α-OH. In addition, the 7α-OH and the 7*β*-OH present in **167** and **168** are not esterified by the enzyme. In both cases, the 3-butanoate is formed also (Figure 23).

Saponification of steroid esters can be steered with *C. cylindracea* lipase (CCL) also.[272,273] This process occurs in the presence of octanol in organic solvents and is characterized by a pronounced stereospecificity and regioselectivity. Thus, the 3α-esters of 3α,17*β*-diacetoxy steroid **169** resisted liberation whereas the 3*β*-isomer **170** is transformed to the corresponding alcohol in 79% yield. The 17α-acetate of 3,17α-diacetoxy estradiol **171** is also saponified but at a slower rate than the 3-acetate (Figure 23). In the case of the androstane derivatives **172** and **175**, different selectivities of *C. antarctica* lipase (CAL) and CCL were observed.[273] Thus, the alcoholysis of **172** in the presence of CAL afforded the C-3 deprotected product in 75% yield whereas CCL led to the removal of the acetate at C-16 in 66% yield. Treatment of **173**, **174**, and **176** with CCL led to cleavage of the C-3 acetate in 79%, 87%, and 83% yields, respectively.[273,274]

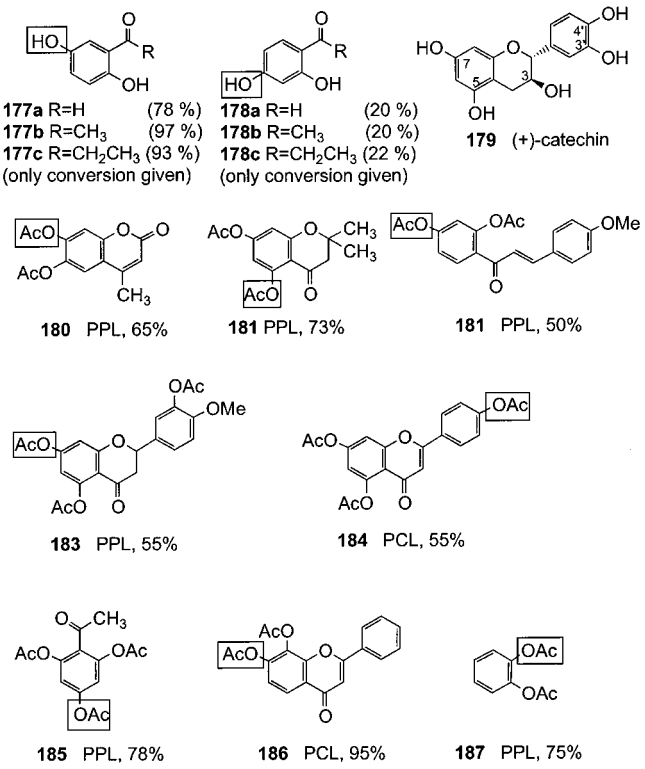



**Figure 24.** Selective enzymatic protection and deprotection of polyphenolic compounds.

## H. Phenolic Hydroxy Groups

Polyphenolic compounds occur widely distributed in nature and may possess a variety of interesting biological properties, e.g., antibiotic, antiviral, and antitumor activity. The synthesis and further elaboration of these compounds often requires the selective protection or deprotection of specific phenolic hydroxy groups. To achieve this goal, the methods highlighted above for the various aliphatic polyols can be applied successfully also.

For instance, for the enzyme-catalyzed acetylation of phenols, first six different lipases were screened for activity.[275,276] Out of these, only the lipase from *C. viscosum* (CVL) showed significant activity. In a subsequent study, the lipase from *P. cepacia* (PSL) turned out to be a more efficient biocatalyst, which was successfully used for the regioselective acylation of various aromatic dihydroxycarbonyl compounds[277] and (+)-catechin.[278] Thus, by using PSL as the biocatalyst, the dihydroxy aldehydes and ketones **177**, **178**, and related compounds were selectively acetylated in conversions ranging from 20% to 97% using vinyl acetate as acyl donor (Figure 24).[277] (+)-Catechin **179** was also subjected to irreversible acyl transfer conditions. In this case, both the 5-, and 7-monoacetates were obtained in 40% and 32% yields, respectively.[278] Interestingly, the inability of the lipase from *A. niger* to acylate aromatic hydroxy groups has consequently been used for the selective acylation of primary aliphatic hydroxy functions in molecules containing both aromatic and aliphatic OH groups.[279] In fact, even PSL preferentially acylates

primary aliphatic hydroxy groups if they exist in the compound.[280]

In the deprotection of peracetylated polyphenolic compounds a somewhat different scheme has emerged. In this field, a broader spectrum of lipases has been successfully used. For instance, the pentaacetyl derivative of catechine **179** was treated with PSL under alcoholysis conditions (THF, *n*-butanol) to give the 3,3′,4′-trisacetate in 50% yield after 12 h.[278] Upon longer treatment with the biocatalyst, the 3-monoacetyl derivative was isolated in 95% yield.

Thus, the coumarin **180**, chromanone **181**, chalcone **182**, flavanone **183**, as well as several flavones, e.g., **183** and **185**, were regioselectively deacylated by employing different lipases in organic solvents (Figure 24). Porcine pancreatic lipase (PPL) predominantly attacks one of the phenolic acetates present in **180**−**183** with good to high regioselectivity and makes the respective selectively protected compounds available in good yields.[281−283] The flavone acetates **184** and **186** can be partially deacylated with high regioselectivity by transesterification using lipase from *P. cepacia* (PSL) and *n*-butanol in THF.[284,285] However, in other cases the positional specificity displayed by the enzyme was less pronounced. This technique has allowed for an efficient construction of a selectively O-methylated flavonoid.[284]

In addition, aryl alkyl ketones which are important starting materials for the synthesis of polyphenolic natural products may be manipulated selectively by making use of an enzymatic saponification.[283,285−287] In general, in these cases the sterically more accessible ester groups are cleaved, as, for instance, in **185**.[285] All of these examples have in common that a carbonyl group is either directly or vinylogously attached to the aryl moiety. Without such a function present in the molecule, the biocatalysts failed to differentiate the ester groups or completely deacylated the substrates. However, by using the lipases from porcine pancreas (PPL) or *C. cylindracea* (CCL) immobilized on microemulsion-based gels, it was possible to monodeacylate resorcinol and related diesters such as **187** in high yields.[158] Alternatively, by using *tert*-butyl methyl ether saturated with water as solvent, it was possible to selectively monodeacetylate diacetoxynaphthalenes.[288] The influence of the solvent was exemplified by switching the solvent system to acetone/buffer. Under such conditions only completely deacylated products were obtained.

## VI. Outlook

During the past decades substantial progress has been achieved in the development of enzymatic protecting group techniques. In particular, it was demonstrated that these methods offer viable alternatives to classical chemical approaches. Not only do the biocatalyzed transformations complement the arsenal of chemically removable protecting groups, in many cases they additionally offer the opportunity to carry out useful functional group interconversions with selectivities which cannot or can only hardly be matched by chemical techniques. However, the overwhelming majority of the investigations carried out in this area has restricted it to the study of the protection and deprotection of model compounds. Complex synthetic schemes were nearly generally avoided. Whereas this appears to be particularly true for the carbohydrate field, noticeable examples which demonstrate the capacity of these biocatalyzed processes were recorded in peptide and peptide conjugate chemistry, i.e., in the synthesis of lipo-, glyco-, and nucleopeptides. The data and observations highlighted above, however, provide a solid basis for the application of biocatalysts in the handling of protecting group problems in complex multistep syntheses.

On the other hand, the use of biocatalysts in protecting group chemistry in the sense of a general method deserves and is certainly awaiting further intensive development. Numerous applications of the known enzymes appear to be possible in all areas of preparative chemistry. In addition, the use of catalytic proteins which have not yet been applied to carry out protecting group manipulations and of biocatalysts unknown today or which will be developed, e.g., by evolutionary approaches, will create new opportunities for improved organic syntheses.

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