Biocatalysis in Polymer-Supported Synthesis: Enzyme-Labile Linker Groups

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Abstract: The development of new and broadly applicable linker groups which are stable under a variety of reaction conditions and allow release of the desired products from polymeric supports under very mild conditions is of great interest for organic synthesis and combinatorial chemistry. In this review we summarize the results of the research activities directed towards the introduction of enzyme-labile linkers which yield a variety of different target molecules through enzymatic cleavage from different polymeric supports.

Keywords: cleavage reactions; combinatorial chemistry; enzyme catalysis; linker groups

1 Introduction

Combinatorial chemistry and parallel synthesis of compound libraries on polymeric supports are efficient methods for the generation of new substances with a predetermined profile of properties.[1–3] The anchoring of one reactant to a polymeric support has the advantage that an excess of reagent may be used, while purification is kept manageable. This is particularly important if the reaction is to be carried out with several reactants in the same reaction vessel. Solid-phase synthesis involves the use of linkers between the compounds to be varied combinatorially and the solid supports which are stable during the reactions. These linkers have to be cleavable as desired, usually at the end of the synthetic sequence, with high selectivity and in good yield, without affecting the structure(s) of the product(s) that are released from the polymeric supports.

Linkers have previously mostly been cleaved by classical chemical methods, for instance, using strong acids. Such conditions often restrict the application of the linkers, i.e., acid-sensitive linkers are not suitable for acid-labile compounds, such as carbohydrates. Specific linkers have therefore been developed for acid-labile compounds, such as silyl ether linkages, thioether linkages,[4] and ester linkages.[5] Although such linkers may be cleaved in the presence of acid-labile groups, they have the disadvantage that they are themselves quite labile to common chemical reagents that one might want to employ on the solid phase. For example, esters and silyl ethers are unstable to base and thioethers are unstable in the presence of oxidants, such as *m*-chloroperbenzoic acid, and to electrophilic reagents, such as alkylating agents.

In organic synthesis, enzymatic methods in many cases have opened up advantageous alternatives to such classical chemical techniques since enzyme-catalyzed transformations typically proceed under very mild conditions (pH 5 – 8, 25 – 37 °C) and with pronounced chemo-, regio-, and stereoselectivity.

In particular, enzymatic transformations have enabled the establishment of alternative protecting group techniques. They were successfully employed for the development of blocking functions which are stable during a wide variety of difficult reactions, yet can be removed under extremely mild conditions.[6–9] Linker groups in principle are polymer-enlarged versions of blocking functions used in regular solution-phase chemistry. Therefore, enzymatic transformations that may be employed for the removal of protecting groups in solution in principle also may open up alternative opportunities to release compounds from polymeric supports.

In this review, we summarize the progress made in this field since its inauguration. We differentiate the linkers as *exo*- and *endo*-linkers cleavable by *exo*- and *endo*-enzymes, respectively (Scheme 1), as proposed by Flitsch et al.[10]

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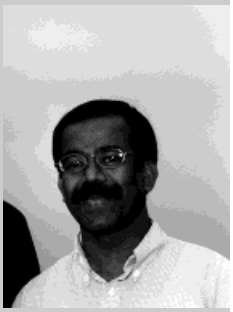
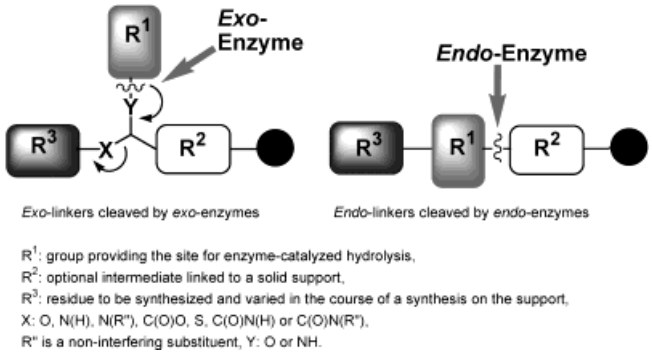
Herbert Waldmann, born in 1957, received his Dr. rer. nat. in 1985 (Universität Mainz, H. Kunz). After postdoctoral studies (1985–1986, Harvard University, George Whitesides) and habilitation (1991, Universität Mainz) he accepted a professorship at the Universität Bonn in 1991. In 1993 he moved to the 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 as 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, of the Carl Duisberg Award of the Gesellschaft Deutscher Chemiker, the Otto-Bayer-Award and the Steinhofer Award of the Steinhofer Foundation. His current research interests include bioorganic chemistry and natural product synthesis as well as biocatalysis, stereoselective synthesis, and combinatorial chemistry. A major focus of the 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 (see the home page for further information: www.mpi-dortmund.mpg.de).

Reinhard Reents was born in Oldenburg (Germany) in 1972. He studied chemistry at the RWTH Aachen and received his diploma degree in 1998. He joined the research group of Professor H. Waldmann at the Universität Karlsruhe in 1998. In 2000, he moved to the Max-Planck-Institut of Molecular Physiology in Dortmund (Department of Chemical Biology). In his graduate studies he is concentrating on the synthesis of fluorescently labeled lipopeptides, their application in studies on biological signal transduction and the combinatorial synthesis of natural product analogues.



D. A. Jeyaraj was born in 1971 in Kumarapuram, Tamilnadu, India. He has completed his B.Sc. in Chemistry at Arignar Anna College, Aramboly, in 1991 and his M.Sc. in Chemistry at Saraswathi Narayanan College, Madurai, in 1993. He obtained his Ph.D. at Indian Institute of Technology, Kanpur, under the guidance of Prof. V. K. Yadav in 1998. He received an Alexander von Humboldt fellowship, Germany, for his postdoctoral study. Currently he is doing his postdoctoral studies with Prof. Dr. H. Waldmann at Max-Planck Insitut für Molekulare Physiologie, Dortmund, Germany. His interests are synthesis of biologically active natural products and physical organic chemistry.



Scheme 1. Graphical representation of *exo*- and *endo*-linkers.

Exo-linkers are composed of three units: i) a group providing the site for enzyme-catalyzed hydrolysis (R^1), ii) a site for attachment of the target molecule (R^3), and iii) a site for attachment to a further optional spacer (R^2).

Endo-linkers are linkers in which the target molecule (R^3), the group, which provides a site for enzyme catalyzed hydrolysis (R^1), and a further optional spacer (R^2) are attached to the polymeric support in a linear arrangement. By means of enzyme-mediated dissection, the target molecule, in many cases tagged with the functional group recognized by the enzyme, is released.

Examples of *endo*-cleavable linkers have been reported (Table 1). However, in many cases the product is tagged with part of the linker. For instance, the *endo*-peptidase chymotrypsin cleaves *endo*-linkers towards the middle of a peptide-chain or "internally". Not only does this limit the methodology to a very small number of enzymes, but it may also restrict the structure of molecules that may be generated. For instance, this method will typically (but not necessarily, see Scheme 2 and Scheme 3) generate compounds containing C-terminal aromatic amino acids, which is necessary for recognition by chymotrypsin.

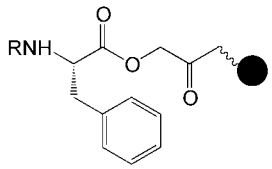
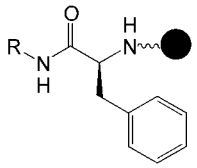
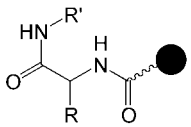
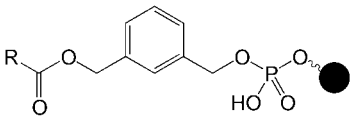
By contrast, *exo*-linkers do not restrict reactant structure and can be cleaved by more readily available *exo*-enzymes, which act at the end of a chain or "externally" (Table 2). Furthermore, *exo*-cleavable linkers yield untagged products upon cleavage from the solid support.

2 *Endo*-Linkers

For a better overview, examples of *endo*-linkers and the enzymes used for the cleavage of the product from the solid phase which have been described in the literature so far are given in Table 1.

Wong et al.[11] introduced a silica-based solid support having a specific enzymatically cleavable linker for the synthesis of glycopeptides and oligosacchar-

Table 1. Examples of *endo*-linkers and respective cleavage enzymes.

Linker	Enzyme	Examples	Ref.
	α-Chymotrypsin	Glycopeptide synthesis	[11]
	α -Chymotrypsin	Oligosaccharide synthesis	[12,13]
	Ceramide glycanase	Oligosaccharide synthesis	[14]
	Phosphodiesterase	Peptide synthesis	[16]

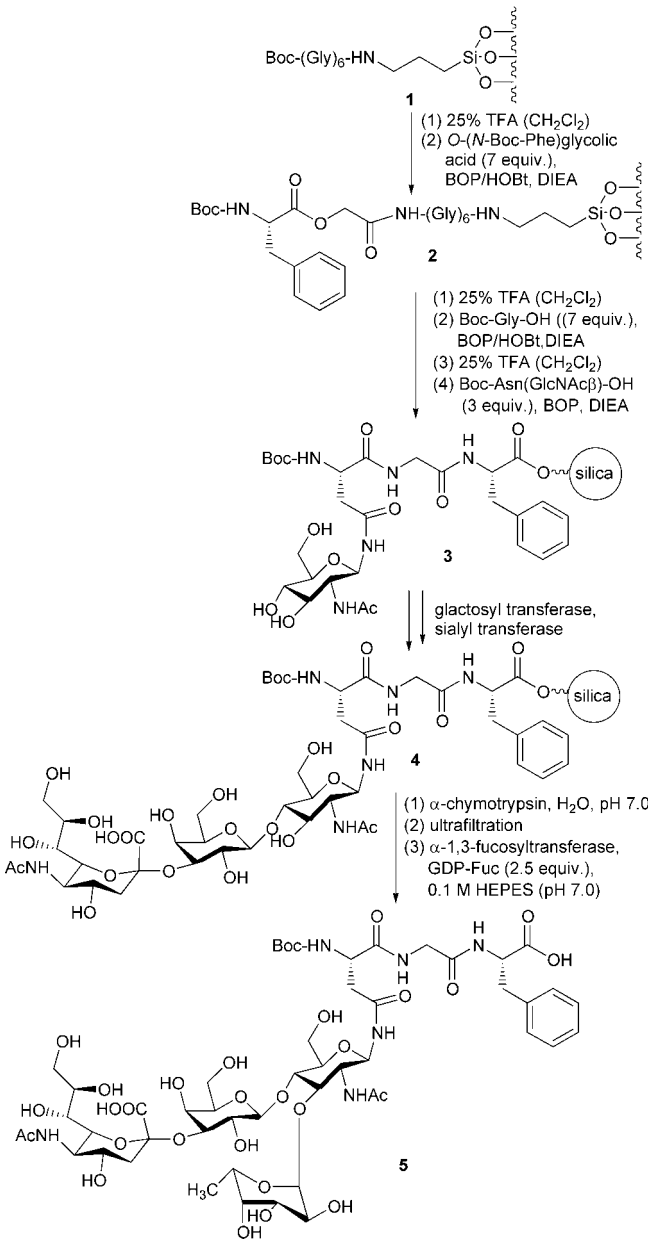
ides. They found that styrene- and sugar-based polymers tend to swell which leads to low coupling yields. Their choice of solid support is aminopropyl silica based on the facts that: (a) it is compatible with both aqueous and organic solvents, (b) it has a large surface area accessible to biomolecules, and (c) it has a sufficient density of functional groups.

A hexaglycine spacer was attached to the solid support to give a substitution of 0.2 mmol/g of dry silica and the excess amino groups were then capped using acetic anhydride. In the next step a selectively cleavable, α-chymotrypsin sensitive, phenylalanine ester **2** was implemented for the release of the products from the solid support under mild conditions. Then it was transformed to **3** followed by reactions with glycosyl transferases to yield **4**. Finally, the desired glycopeptide was cleaved from the solid support in high yield by treatment of **4** with α-chymotrypsin (Scheme 2).

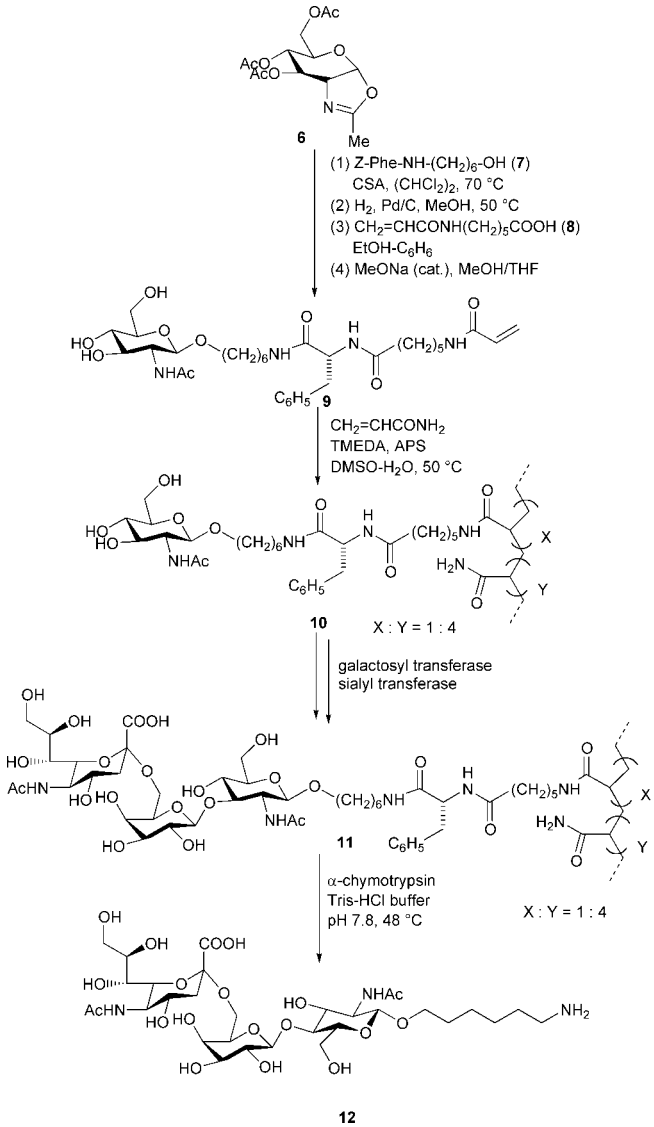
Nishimura et al.[12,13] described a novel method for the enzymatic synthesis of oligosaccharide derivatives employing an α-chymotrypsin-sensitive linker. The synthesis of the water-soluble GlcNAc-polymer **10**, sensitive to α-chymotrypsin, is shown in Scheme 3. Oxazoline derivative **6** was coupled with 6-(*N*-benzyloxycarbonyl-L-phenylalanyl)amino-1-hexanol (**7**) followed by *N*-deprotection of the phenylalanine and subsequent condensation with 6-acrylamidocaproic acid (**8**). De-*O*-acetylation gave the polymerizable GlcNAc derivative **9**. Finally, co-polymerization of acrylamide and monomer **9** in the presence of ammonium persulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) gave the polymer **10** in high yield. The polymer **10** was then subjected to galactosylation and subsequent sialylation with the corresponding glycosyl transferases to yield **11**. The final product **12** was cleaved from the water-soluble support by treatment with α-chymotrypsin at 40 °C for 24 h in 72 % overall yield from **10**.

Nishimura and Yamada[14] introduced a water-soluble polymeric support having a linker recognized by ceramide glycanase for a synthesis of ganglioside GM3 (**17**). The synthesis of the polymerizable lactose derivative **14** with a ceramide glycanase-sensitive linker is shown in Scheme 4. The lactosylceramide (LacCer) mimetic glycopolymer **15** is obtained from the monomeric precursor **14** by co-polymerization with acrylamide.

This solid support **15** was converted to the intermediate product **16** by sialylation using βGal1→3/4GlcNAc α-2,3-sialyltransferase. Finally, the polymeric support was cleaved by transglycosylation with leech ceramide glycanase in the presence of excess ceramide as acceptor to give the desired product **17** in high yield (Scheme 4). An advantage of the water-soluble polymer is that the transformation can be



Scheme 2. Glycopeptide synthesis and α-chymotrypsin-catalyzed release from the solid support.

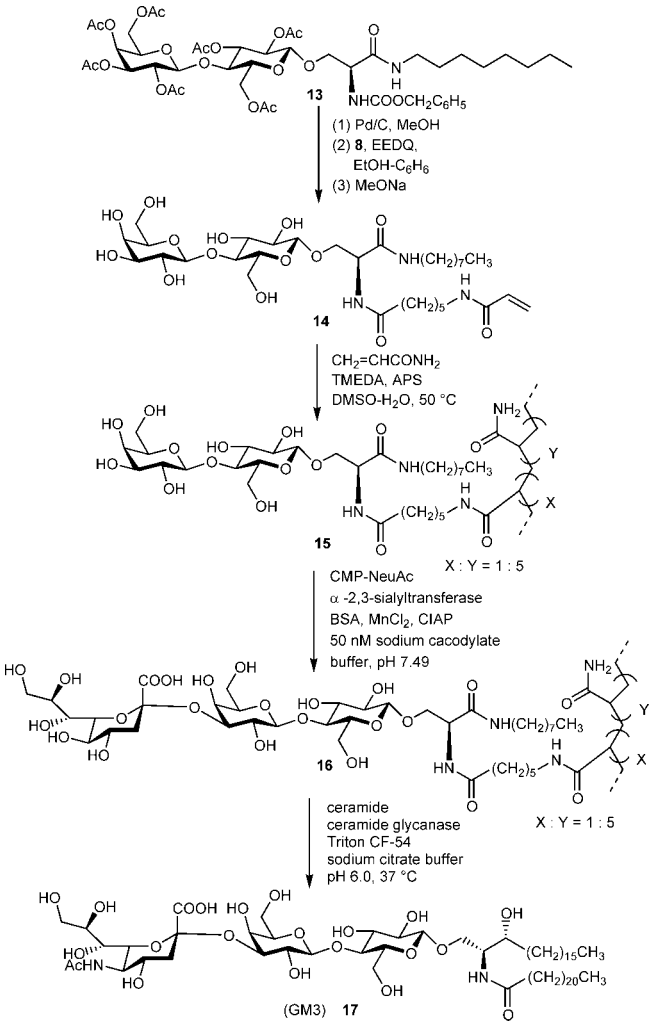


Scheme 3. Oligosaccharide synthesis and α-chymotrypsin-catalyzed release from the solid support.

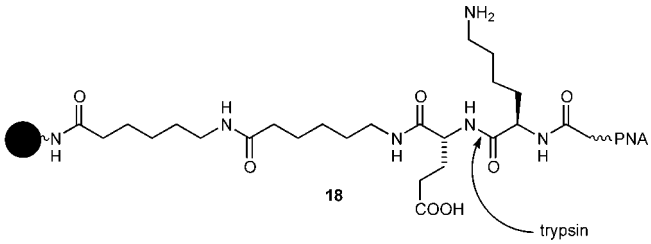
monitored by NMR spectroscopy during the enzymatic glycosylation steps.

Arrays of up to 1000 PNA oligomers of different sequence were synthesized by Jensen et al. on polymer membranes (Scheme 5).[15] The PNA chain was linked to the peptide spacer glutamic acid-(γ-*tert*-butyl ester)-(ε-aminohexanoic acid)-(ε-aminohexanoic acid) [Glu[O*t*Bu]-εAhx-εAhx] via an enzymatically cleavable Glu-Lys handle. The Glu[O*t*Bu]-εAhx-εAhx spacer was coupled to the amino-functionalized membrane by standard Fmoc-chemistry. Then the membranes were mounted in an ASP 222 Automated SPOT Robot and a grid of the desired format was spotted at each position. The free amino groups outside the spotted areas were capped and further chain elongation was performed with Fmoc-protected PNA monomers to synthesize the desired PNA oligomers. After completion of the synthesis, the PNA oligomers were cleaved from the solid support by incubation with bovine trypsin solution in ammonium bicarbonate at 37 °C for 3 h.

One of the very first papers concerning *endo*-linkers was published by Elmore et. al (Scheme 6).[16] They described a new linker containing a phosphodiester group for solid-phase peptide synthesis using a Pepsyn K (polyacrylamide) resin. After completion of coupling and deprotection cycles, the phosphodiester **20** was cleaved with a phosphodiesterase. In this way β-casomorphin, Leu-enkephalin, and a collagenase substrate were synthesized in high yields.



Scheme 4. Ceramide glycanase-mediated release by transglycosylation.

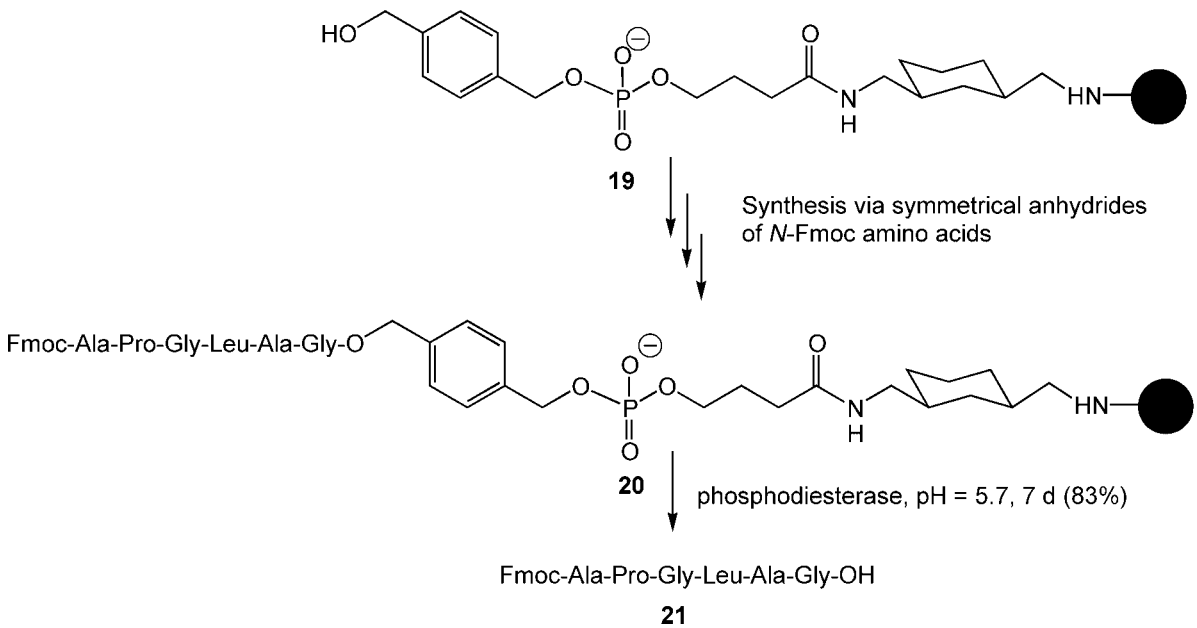


Scheme 5. Trypsin-mediated cleavage of a peptide bond in PNA oligomer synthesis.

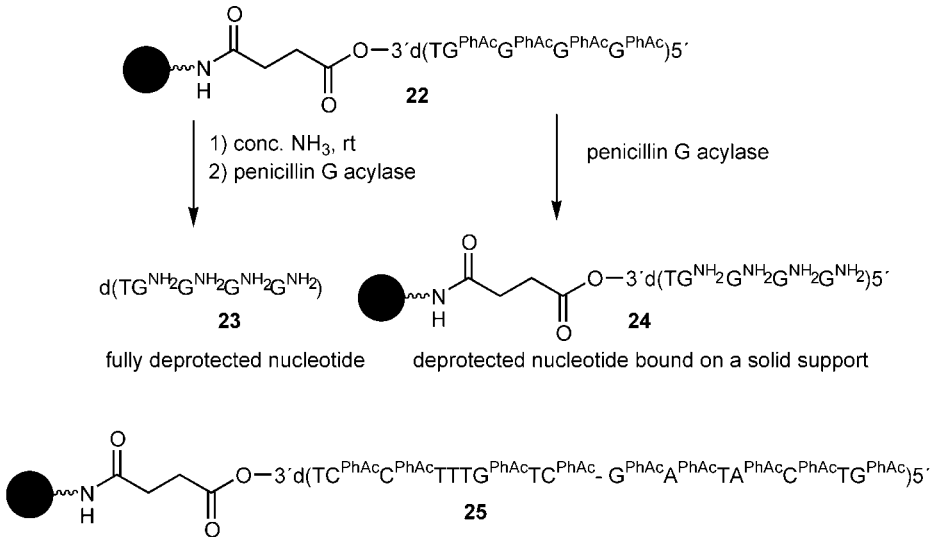
In the context of enzymatic cleavage of linkers on polymeric supports, particular attention was paid to the general question whether enzymatic transformations on resins are viable and high-yielding. An in-depth treatment of this problem is beyond the scope of this review. However, a few examples for the application of biocatalyzed transformations on solid supports shall serve to illustrate that such transformations can indeed be employed advantageously for various purposes.

For example, the phenylacetamide (PhAc)-protected oligonuceotides **22** and **25** were synthesized on a solid carrier and cleaved off without deblocking the nucleobases (Scheme 7).[17] Release of all PhAc groups from the penta- and hexadecanucleotides was achieved by penicillin G acylase treatment at pH 7 and room temperature. Furthermore, the enzyme even deblocked solid phase-anchored oligonucleotides, such as **22**, on the carrier without attacking the linker groups.

Meldal et al. described the proteolytic cleavage of the alanine-tyrosine bond in a resin-bound decapeptide by treatment with the 27 kDa protease subtilisin BNP' to demonstrate the accessibility of the interior



Scheme 6. Synthesis of a collagenase substrate on a phosphodiesterase-scissile linker.



Scheme 7. Enzymatic deblocking of the amino groups of oligonucleotides in solution on a solid phase [controlled pore glass (CPG) beads, which are represented by spheres].

of the newly designed SPOCC–resin[18] to enzymes.[19]

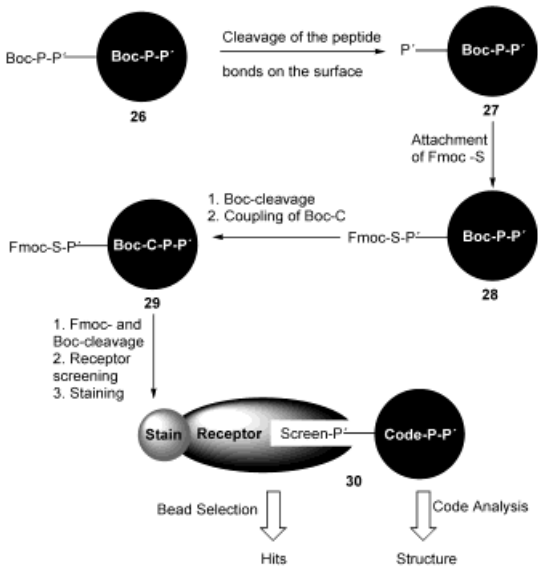
Furthermore, enzymatic hydrolysis of model isopeptides N^{ε}-oligo(L-methionyl)-L-lysine from Biobeads[20] by pepsin, chymotrypsin, cathepsin C (dipeptidyl peptidase IV), and intestinal aminopeptidase N was investigated using high-performance liquid chromatography to identify and quantify the hydrolysis products.[21]

Larsen et al. reported the enzymatic cleavage of a desB30 insulin B-chain from a presequence $[Lys(Boc)]_6$. This spacer shifts the conformation of the growing peptide chain from a β-structure to a random coil conformation and reduces peptide-chain aggregation, which otherwise causes serious synthetic problems. Novasyn KA[22] was employed as a solid support, unfortunately, no information about the enzyme used was reported.[23]

Barany and Lebl et al. were the first to exploit the different enzyme accessibility of surface and interior areas of a given bead and the resulting differentiated bead was used to simultaneously synthesize a peptide library on the surface and code for this on the interior.[24]

This clever strategy is illustrated in Scheme 8. Selective cleavage of short N^{α}-protected peptide substrates with chymotrypsin from the surface area of TentaGel-AM-beads **26** leaves the majority of the peptide attachment sites in the interior uncleaved to afford **27** ("shaving" methodology). The first residue is attached using orthogonal Fmoc-chemistry to provide **28**. Coding is done by using standard Boc-chemistry on the interior of the bead to yield **29**. Repetition of this process furnishes a surface peptide, which is encoded internally (**30**).

This generation of two structures on the same bead allowed the investigation of the synthesized peptide



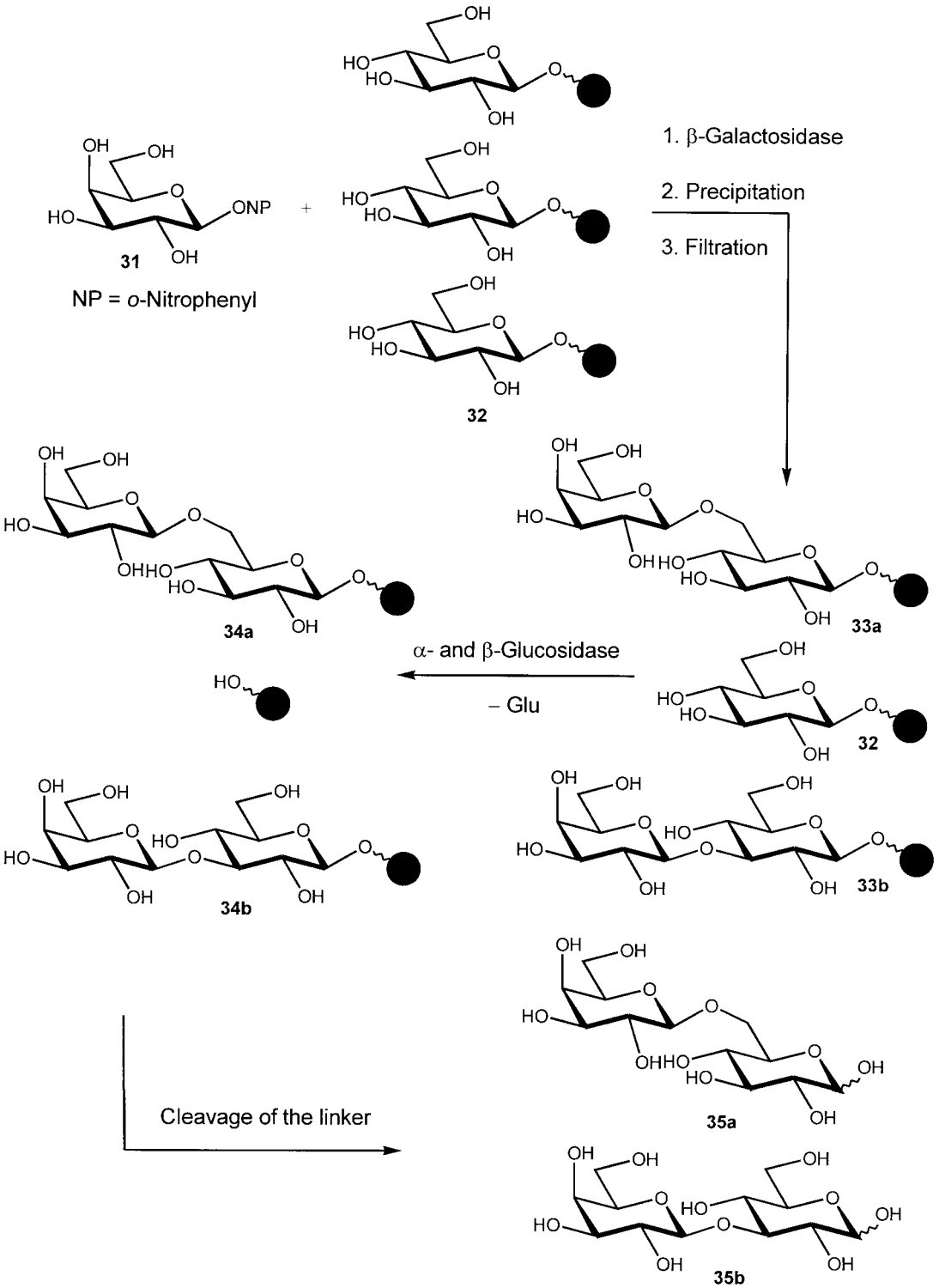
Scheme 8. Peptide encoded combinatorial peptide libraries via enzyme- mediated spatial segregation. P-P': substrate with a scissile bond between P and P'; S: terminal residue of the screening structure; C: terminal residue of the coding structure.

library (1×10^5 members) with different receptors (anti-β-endorphin antibody, streptavidin, and thrombin). After the staining procedure was carried out, the beads that showed a color were selected for sequencing and the coding peptides present within the bead were used to deduce the binding structures. This screening led to the discovery of a new thrombin ligand, which binds with an affinity one order of magnitude higher than the natural motif.

Fernandez-Mayoralas et al.[25] used the high substrate specificity of enzymes in their synthesis of ga-

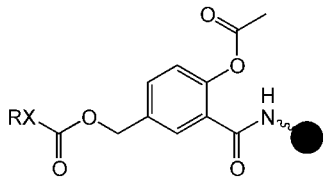
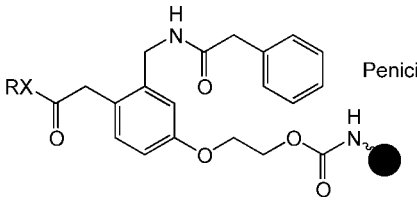
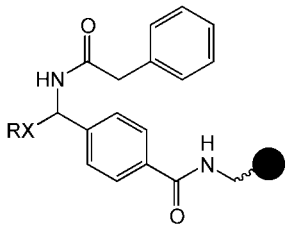
lactose-glucose disaccharides (**35**) on an MPEG-support.[26] After galactosylation of glucose immobilized to the soluble support (**32**) using β-galactosidase, the unreacted monosaccharide glucose was removed by the combined use of α- and β-glucosidases to obtain only MPEG-bounded disaccharides (**33**, Scheme 9). Finally, the obtained disaccharides **35** were released from the support by ethanolysis.

Reetz et al. described the solid-phase enzymatic synthesis of oligonucleotides on Kieselguhr-PDMA-resins via T4 RNA ligase. Concomitantly, they found that RNase A selectively cleaves the last bound nu-



Scheme 9. General strategy for the liquid-phase synthesis of disaccharides using glycosidases.

Table 2. Examples of *exo*-linkers and respective cleavage enzymes.

Linker	Enzyme	Examples	Ref.
	Lipase	Pictet-Spengler reaction, nucleoside immobilization	[30]
	Penicillin acylase	Palladium cat. C-C-couplings, Mitsunobu- and Diels-Alder reactions, 1,3-dipolar cyclo-additions	[37,38]
	Penicillin acylase	Immobilization of alcohols (e.g. Fmoc-protected serine methyl ester, glycosides) and amines (e.g. phenyl-alanine)	[28,29]

cleotide at the ribose sugar leaving a 3',5'-diphosphorylated oligomer behind on the resin, but application in synthesis has not yet been undertaken.[27]

3 *Exo*-Linkers

An *exo*-linker according to Scheme 1 must contain an enzyme-labile group R^1, which is recognized and attacked by the biocatalyst. Possible combinations could be: phenylacetamide/penicillin amidase, ester/esterase, monosaccharide/glycosidase, phosphate/phosphatase, sulfate/sulfatase, and peptides/peptidases.[28] Until now only the following systems have been worked out (Table 2).

In independent and simultaneous investigations Flitsch et al.[28,29] and Waldmann et al.[30,31] developed a selectively cleavable *exo*-linker, which can be cleaved with penicillin G acylase, a commercially available and widely used enzyme.[17]

Penicillin acylase catalyzes the hydrolysis of phenylacetamides and has been used in peptide synthesis for the cleavage of protecting groups.[32] In linker **36** developed by Flitsch et al.[28,29] (Scheme 10) –XR represents the alcohol or amine group of the target molecule. Hydrolysis of the phenylacetamide moiety generates the hemiaminal **38** which readily fragments in aqueous medium and thereby releases the desired products RXH.

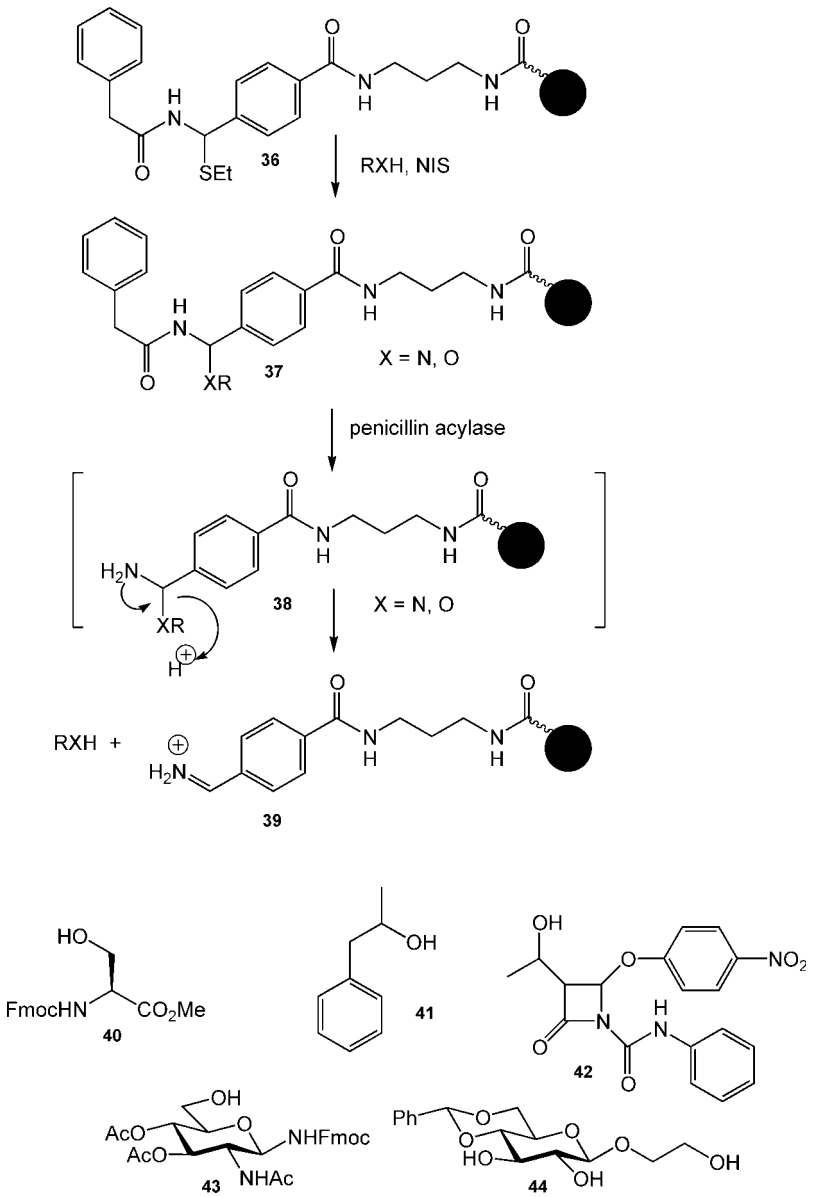
The thioethyl group present in the anchor group of **36** was activated by treatment with *N*-iodosuccinimide (NIS) followed by displacement with a variety of alcohols (**40** – **42**). To prove the possible application of this linker in solid-phase carbohydrate synthesis, protected glycosides **43** and **44** were coupled to linker **36** and released enzymatically.

Flitsch et al. also describe the immobilization and enzymatic cleavage on a variety of amines.[28] Nevertheless, the application of this enzyme-labile linker group in multi-step syntheses on the solid phase and subsequent enzyme-initiated release from the polymeric support has not been described yet.

Waldmann et al. described the *exo*-linker **45**.[30,31] The anchor group comprises a 4-acyloxy-3-carboxybenzyloxy group, which is recognized and attacked by the biocatalyst, so that a spontaneously fragmenting intermediate is generated, thereby releasing the desired compound (Scheme 11).[33–35]

The linker **45** is attached as an amide to the solid phase. Cleavage of the acyl group by a lipase generated a phenolate **46**, which fragments to give a quinone methide **47** and releases the product **48**. The quinone methide remains on the solid phase and is trapped by water or an additional nucleophile.

Following this cleavage principle amines (bound as urethanes), alcohols (bound as carbonates), and carboxylic acids (bound as esters) can be detached from the polymeric carrier. The substrate specificity of the



Scheme 10. Loading and cleavage of a penicillin acylase scissile linker.

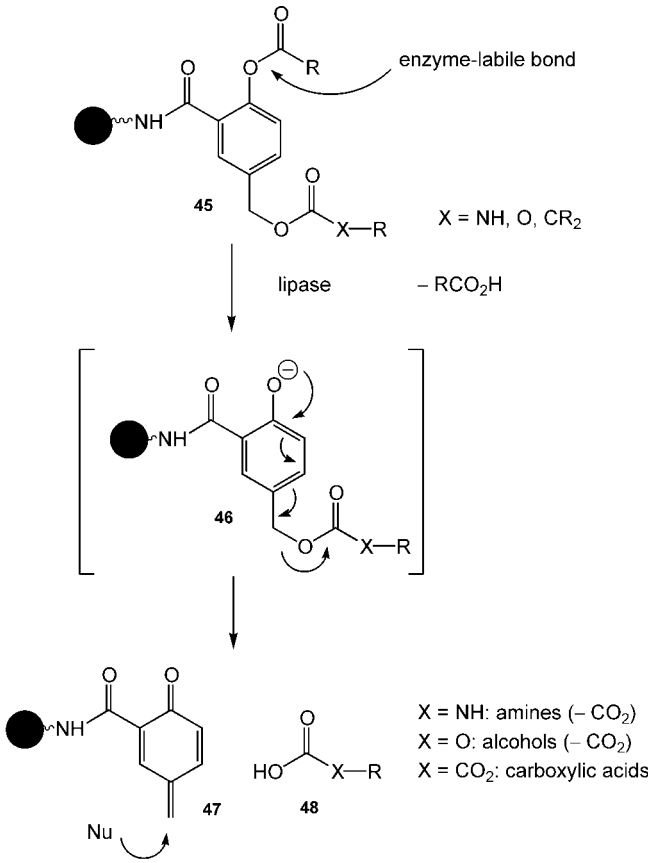
enzyme guarantees that only the intended ester is cleaved.

As the polymeric support TentaGelS-NH_2 was chosen, i.e., a polystyrene resin equipped with terminally NH_2-functionalized oligoethylene glycol units. It has a polar surface and swells in aqueous solutions allowing the biocatalyst access to the polymer matrix.[36]

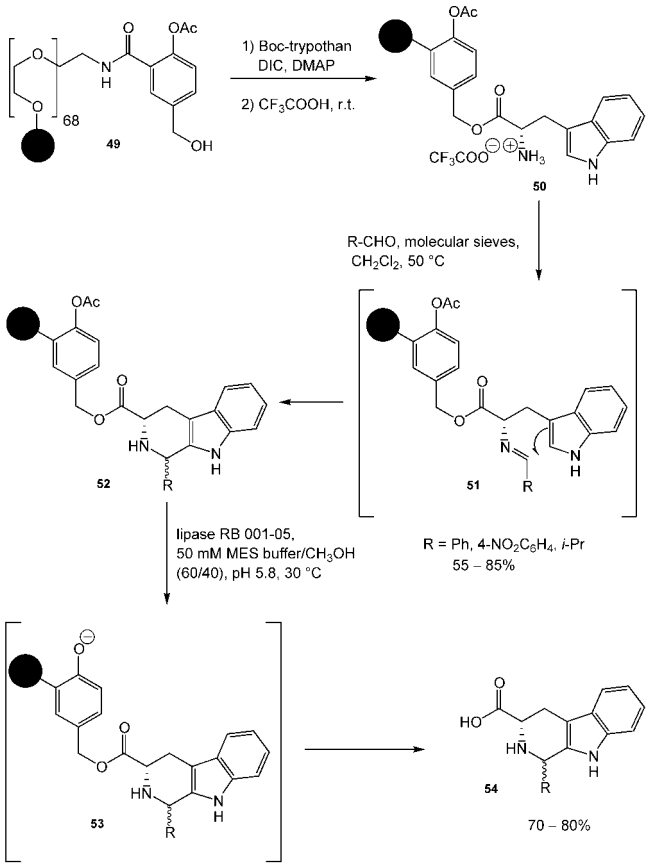
The applicability of the enzyme-labile anchor group was demonstrated by the synthesis of tetrahydro-β-carbolins **54** employing the Pictet-Spengler reaction (Scheme 12).

The benzylic alcohol group of the linker **49** was first esterified with Boc-L-tryptophan, and after its N-terminal deprotection the support-bound tryptophan **50** was reacted with aliphatic and aromatic aldehydes to give imines **51**, which cyclized immediately in reasonable to high yields to the tetrahydro-β-carbolins **52**. Lipase RB 001–05 selectively attacked the acetate incorporated into the linker and generated the corresponding phenolate **53**, which then fragmented spontaneously. Following these multi-step transformations the desired tetrahydro-β-carbolins **54** were obtained in 70 – 80% yield.

Waldmann et al. developed a second *exo*-linker following a new approach[37,38] which makes use of a safety-catch linker. It is based on the enzymatic cleavage of a functional group embodied in the linker. Thereby an intermediate is generated, which subsequently cyclizes intramolecularly according to the principle of assisted removal[39–43] and thereby releases the desired target compounds (Scheme 13).



Scheme 11. Principle for the development of the enzyme-labile 4-acyloxybenzyloxy linker group.



Scheme 12. Solid-phase synthesis of tetrahydro-β-carbolins and subsequent detachment by enzyme-initiated fragmentation of the anchor group.

The linker group is immobilized as a urethane to the amino-functionalized carrier **55**. It facilitates the attachment of a variety of molecules such as alkyl halides, alcohols, or amines bound as carboxylic acid esters and amides.

According to the safety-catch principle, the separation of the desired products proceeds in a two-step process. First, penicillin G acylase hydrolyzes the phenylacetamide with complete chemo- and regioselectivity and under exceptionally mild conditions (pH 7.0, room temperature or 37 °C).[44–46] Then the generated activated intermediate, i.e., benzylamine **56,** cyclizes to polymer-bound lactam **57** and releases the desired target molecule **58**.

As polymeric support POE 6000 was used, a soluble polyethylene glycol derivative functionalized at both termini with an amino group and with an average molecular mass of 6000 Da.[47,48] After completion of the homogeneous reactions it can be precipitated, filtered off, and washed with diethyl ether, thereby facilitating the separation of surplus reagents and the side products. Furthermore it allows for NMR spectroscopic monitoring of the reactions.[49]

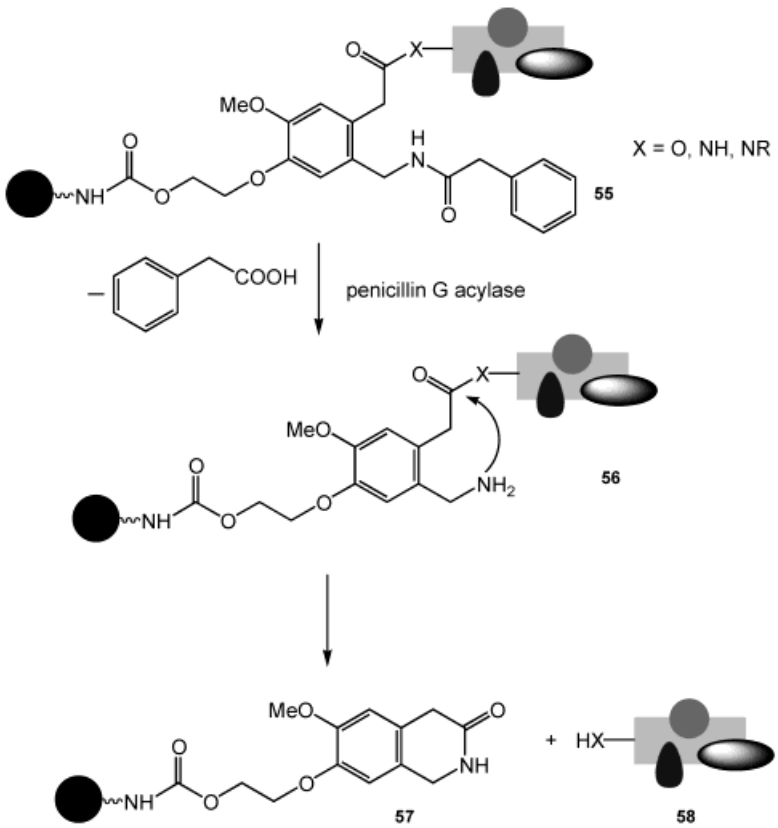
Most importantly, it is soluble in aqueous solutions, thereby allowing efficient access of the enzyme to the polymer-fixed linker group.

The suitability of the polymer-linker conjugate was examined for a variety of transformations, in particular Pd(0)-catalyzed reactions. For instance, the polymer-bound aryl iodide **62** was transformed quantitatively in a Heck reaction to a cinnamic acid ester **63** and, to biphenyls **64** and **65** in Suzuki reactions. It gave an alkyne **61** in a Sonogashira reaction and an enol ether **59** in a Stille reaction (Scheme 13).

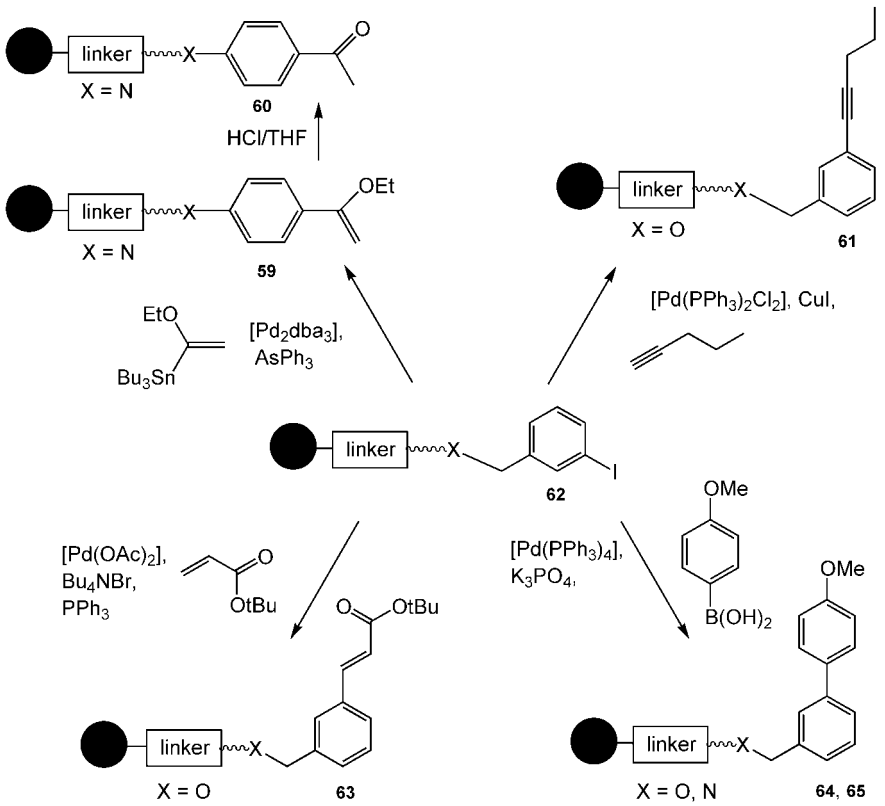
The desired benzyl alcohols or amines were released by incubation of the corresponding polymer conjugates **60**, **61**, **63 – 65** with penicillin G acylase at pH 7 and 37 °C in high yields and isolated with a purity of >95% by simple extraction with diethyl ether. In the case of the amines (X = N), the expected lactam was formed upon warming of the reaction mixture to 60 °C.

Furthermore, the applicability in a Mitsunobu reaction esterification and a Diels–Alder reaction was proven (Scheme 15). The polymer-bound benzyl alcohol **66** was reacted with 4-acetamidophenol in the presence of the Mitsunobu reagent to give phenyl ether **67** in quantitative yield. It was released from the polymeric support in high yield.

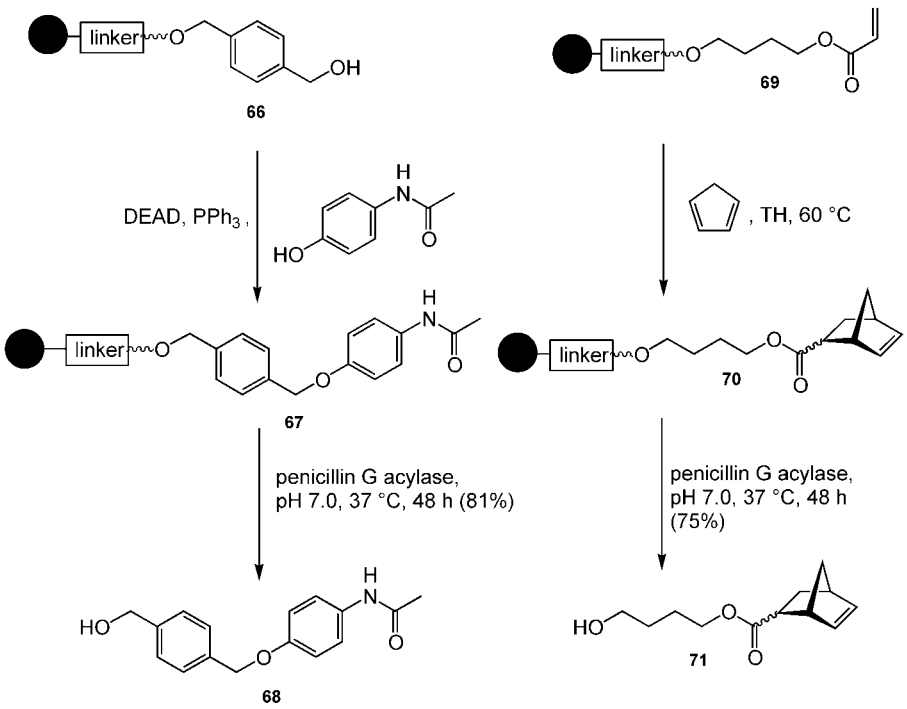
For the Diels–Alder reaction, polymer-bound acrylic acid ester **69** was treated with cyclopentadiene. The



Scheme 13. Principle of the enzyme-labile safety-catch linker.



Scheme 14. Pd(0)-catalyzed reactions on enzyme labile linker-conjugates.



Scheme 15. Mitsunobu and Diels–Alder reactions on enzyme-labile linker-conjugates.

cycloaddition product **70** was formed with an *endo/exo* ratio of 2.5:1 and with quantitative conversion. The subsequent enzymatic release delivered the corresponding alcohols (**68, 71**) in high yield and purity.

4 Conclusion and Outlook

In this review the successful application of enzyme-labile linker groups in polymer-supported synthesis has been summarized. A key goal of combinatorial chemists is to identify mild synthetic methods for the cleavage of compound libraries from the solid-phase ideally at room temperature and under neutral conditions. The development of enzyme-labile linkers may prove to be an important step in this direction. These linkers allow the synthesis of numerous different classes of compounds such as peptides, glycopeptides, oligosaccharides, PNAs, oligonucleotides, and various further natural products as well as numerous pharmacologically relevant compound classes, e.g., different heterocycles, bicyclic compounds, etc. on different polymeric supports.

These results prove that enzymes, in general, may be valuable reagents for carrying out transformations on polymeric supports. The particular strength of enzyme-labile linker groups and the corresponding use of biocatalysts for releasing the target compound are the mildness of the reaction conditions and the pronounced selectivity of the enzymes. Thus, such linker groups may prove to be particularly efficient tools in the synthesis of sensitive and multifunctional compounds, carrying already several different protecting groups. In addition, the use of enzymes in combinatorial chemistry and parallel synthesis, in general, may open up viable and efficient alternatives to established classical chemical methods and add to the arsenal of synthetic methods accessible to the chemist.

For instance, one of the emerging trends in compound library synthesis is the use of polymer-fixed reagents.[50–52] Many enzymes have been immobilized on polymeric supports and have been applied as such polymer-fixed catalysts in innumerable cases in research and industry.[53] So why should one not use them together with other polymer-fixed reagents in the preparation of compound libraries as well?

In the application of soluble biocatalysts for carrying out reactions on polymer-bound compounds, however, several important questions remain to be addressed convincingly before the technique will be applied widely. In our opinion the two most urgent ones are 1) What is the best type of polymer compatible with the specific requirements of biocatalysts (for approaches to this problem see also[54])? 2) Do enzymes (or "which enzymes") have sufficient access to the interior of polymer beads to assure efficient release of the synthesized compound?[37]

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