Enzymatically cleavable linker groups in polymer-supported synthesis

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Access to broadly applicable linker groups that are stable under a variety of reaction conditions and enable the release of target compounds from polymeric supports under the mildest conditions is a major goal in combinatorial chemistry. Here, we summarize the development of enzymatically cleavable linker groups used to prepare a variety of different target molecules on polymeric supports.

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▼ The use of polymeric supports in organic synthesis has increased significantly over the past decade [1–7]. This methodology has several advantages, and the three main factors that contribute to the popularity of the technique are:

(1) Reactions are feasible by means of simple operations, that is, the addition of reagents, washing and, finally, cleavage from the resin. This facilitates the development of automated procedures.
(2) Purification steps are eliminated during a multistep synthesis: only the final product of cleavage needs to be purified.
(3) High concentrations of reagents can be used to drive reactions to completion.

However, several important parameters need to be defined for a successful solid-phase synthesis, including the correct choice of solid support and the mode of attachment and cleavage of materials from the resin matrix. Efficiency in anchoring and removing from an adequate linker system relies on the correct choice of linker group: this is crucial when planning a synthetic strategy.

The resin–(spacer)–linker unit should be chemically stable during synthesis and cleavage and, thus, can be considered to be an insoluble, immobilizing protecting group for solid-phase synthesis. Although numerous linkers have been developed over the past 15 years, many have the disadvantage that they are often themselves labile to common chemical reagents that are used in solid-phase synthesis. However, an alternative solution has been developed that is inspired by the successful generation of enzymatically cleavable protecting groups.

Enzyme-labile linkers can be cleaved under advantageous and characteristically mild reaction conditions because enzymatic transformations often occur at pH 6–8, and between room temperature and 40°C. In addition, enzymes often combine a high chemo-, regio- and stereoselectivity for the recognized substrates, with a tolerance for secondary structures.

This review compares different enzyme-labile linkers based on the nature of the hydrolase used [8]. We focus, in particular, on proteases, glycosidases, esterases and amidases.

Enzymatic cleavage

Proteases

Proteases such as chymotrypsin, trypsin and subtilisin [9–13] were investigated to cleave selectively enzyme-scissile linker groups.

α-Chymotrypsin was used by Wong *et al.* to cleave glycopeptides or oligosaccharides from a solid support at pH 7.0 [14]. This protease recognizes phenylalanine and cleaves a corresponding ester bond of a hexaglycine spacer selectively in high yield (Fig. 1).

Enzymatic synthesis of oligosaccharide derivatives using an α-chymotrypsin-sensitive linker has also been described by Nishimura *et al.* [15,16]. The required trisaccharide **3** (Fig. 2) was synthesized on a water-soluble solid support, by an enzymatic transformation with galactosyl and sialyl-transferases. Finally, the oligosaccharide **4** (Fig. 2) was cleaved from the solid support by α-chymotrypsin.

Hoheisel *et al.* used a polymer membrane as the solid support in the synthesis of arrays of up to 1000 peptide nucleic acid (PNA) oligomers, each of different sequence [17]. A trypsin-sensitive Glu–Lys handle was used

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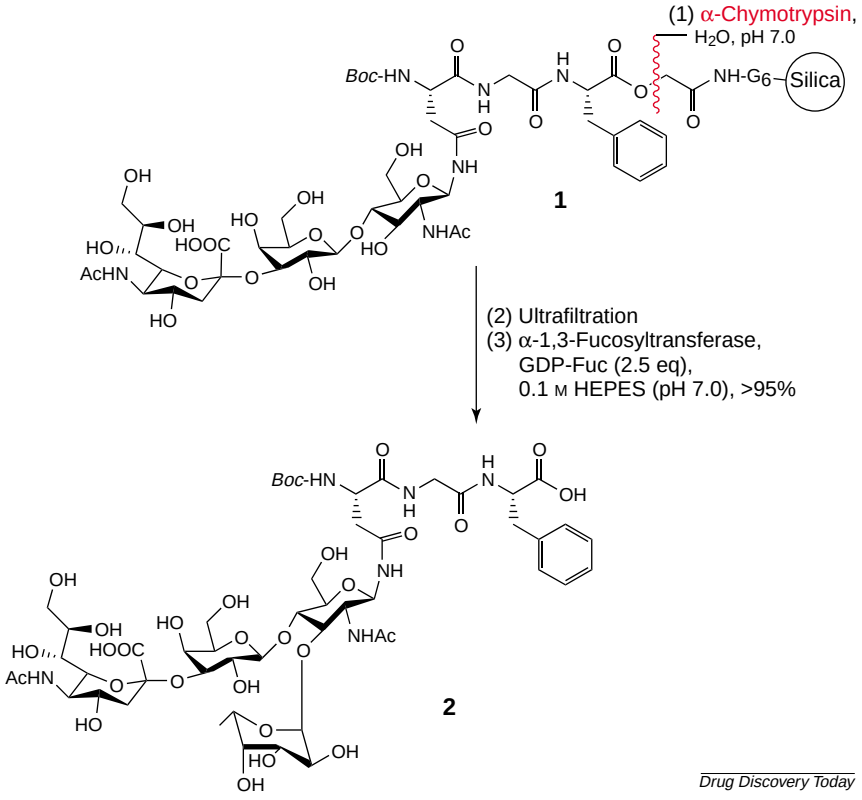

Figure 1. α-Chymotrypsin catalyzed release of glycopeptides from the solid support.

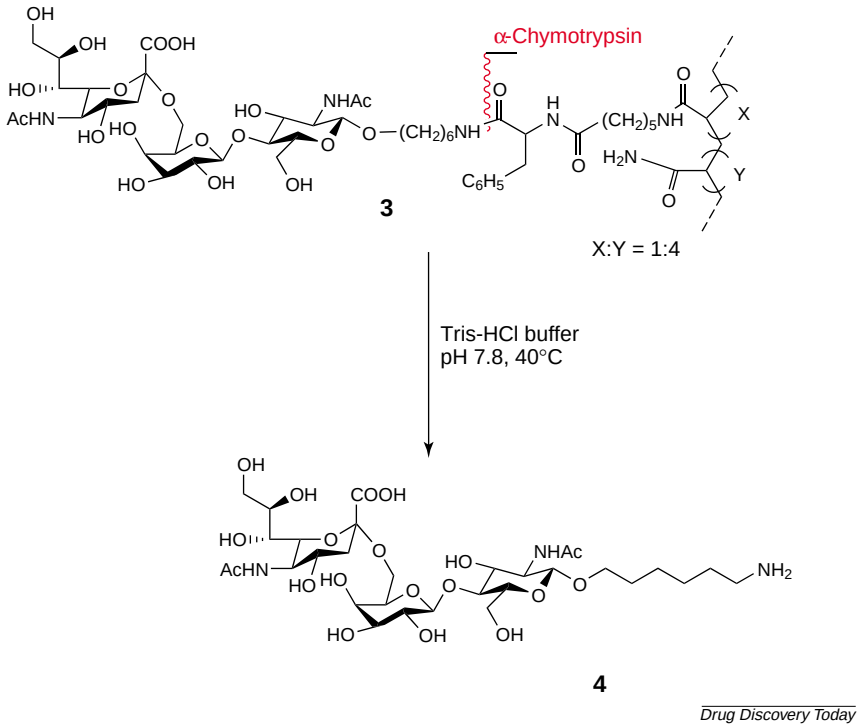

Figure 2. α-Chymotrypsin catalyzed release of oligosaccharides from the solid support.

between the membrane and the PNA oligomer (Fig. 3). The membrane was then mounted onto an ASP 222 automated SPOT Robot (Abimed, Langenfeld, Germany) and a grid of the desired format was spotted at each position. After the PNA oligomers had been synthesized, they were cleaved from the membrane by incubation with bovine trypsin at the Glu–Lys handle (Fig. 3).

Glycosidases

Glycosidases, such as ceramide glycanase, galactosidase [18] and the nuclease, Rnase A [19], have been tested for their ability to cleave a specifically designed linker from a polymeric support. Ceramide glycanase was introduced by Nishimura and Yamada for the synthesis of ganglioside GM3 [20]. After synthesis of trisaccharides **6** (Fig. 4) by enzymatic tranformation, the final product, GM3 (Fig. 4, structure **7**), was cleaved from the solid support by transglycosylation with leech ceramide glycanasein with a yield of 61% (Fig. 4).

Esterases

A phosphodiesterase was the first type of esterase that was tested to cleave linker groups on a solid support. In 1992, Elmore *et al.* described a linker containing a phosphodiester group for solid-phase peptide synthesis using a Pepsyn K (polyacrylamide) resin (Fig. 5) [21]. The collagenase substrate **9** (Fig. 5) was released from the solid support using a calf spleen phosphodiesterase.

Waldmann *et al.* described a linker group **10** (Fig. 6) comprising a 4-acyloxy-3-carboxybenzyloxy moiety, which is recognized by a specific lipase [22,23]. The generated phenolate breaks up to give a quinone methide (Fig. 6, structure **13**) and releases the desired target (Fig. 6, structure **12**).

The reliability of the scissile linker group was scrutinized in the synthesis of tetrahydro-β-carbolins (Fig. 7, structure **19**) using the Pictet–Spengler reaction.

After esterification of the benzylic alcohol group of the anchor **14** with *Boc*-L-tryptophan and its N-terminal deprotection, the immobilized amino acid (Fig. 7, structure **15**) was reacted with aliphatic and aromatic aldehydes to provide imine (Fig. 7, structure **16**). Spontaneous cyclization gave tetrahydro-β-carbolins (Fig. 7, structure **17**), which were detached from the solid support using lipase (RB 001–05; Recombinant Biocatalysis, Diversa, San Diego, CA, USA).

Amidases

Penicillin G acylase catalyzes the hydrolysis of phenylacetamides, and has been used in peptide synthesis for the cleavage of protecting groups [24]. Independently, Flitsch *et al.* [25,26] and Waldmann *et al.* [27,28] have developed two amidase-scissile linker groups.

In the linker developed by Flitsch *et al.*, alcohols or amines are immobilized onto the solid support (Fig. 8) [25,26]. Cleavage of the penicillin acylase scissile anchor group at the phenylacetamide moiety gives the hemiaminal **21** (Fig. 8), which spontaneously fragments in the buffered solution, thereby releasing the desired products RXH (Fig. 8, structures **23–27**).

Although glycosides **26** and **27** were coupled to linker **20** (Fig. 8) and released enzymatically, the suitability of this linker group in carbohydrate-syntheses in solid phase, and subsequent enzyme-initiated release from the polymeric support, has not yet been described in the literature.

Waldmann *et al.* [27,28] prepared a linker by using the safety-catch approach based on penicillin G acylase-catalyzed cleavage of the phenylacetamide moiety within the linker. Treatment of the polymer-bound substrate **28** (Fig. 9) with penicillin G acylase, therefore gives the activated intermediate, benzylamine, which then undergoes intramolecular cyclization, releasing the desired target compounds (Fig. 9).

Several palladium-catalyzed reactions have been tested to investigate their applicability and tolerance to a variety of reaction conditions. The immobilized aryl iodide **32** (Fig. 10) was transformed to either a cinnamyl ester derivative **33** via a Heck reaction, or to biaryl derivatives **35** by Suzuki coupling. Similarly, a Sonogashira reaction yielded alkyne **34** (Fig. 10). After incubation of polymer conjugates **33–35** (Fig. 10) with penicillin G acylase at pH 7.0 and

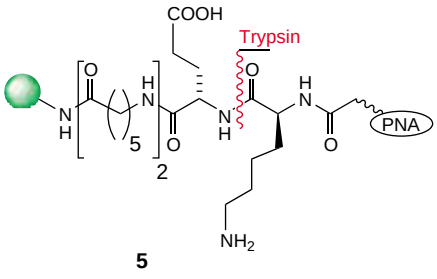

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Figure 3. Trypsin-mediated cleavage of a peptide bond in peptide nucleic acid (PNA) oligomer synthesis.

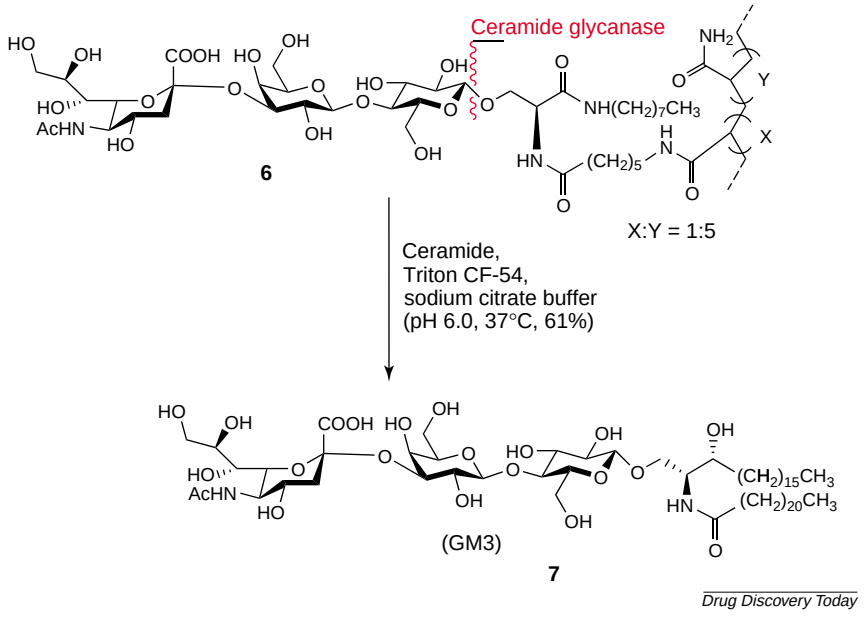

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Figure 4. Ceramide-glycanase-mediated release of GM3 by transglycosylation.

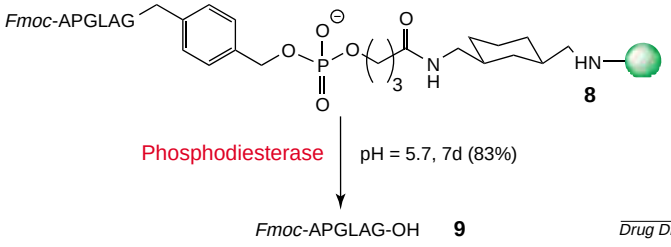

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Figure 5. Synthesis of a collagenase substrate on a phosphodiesterase-scissile linker.

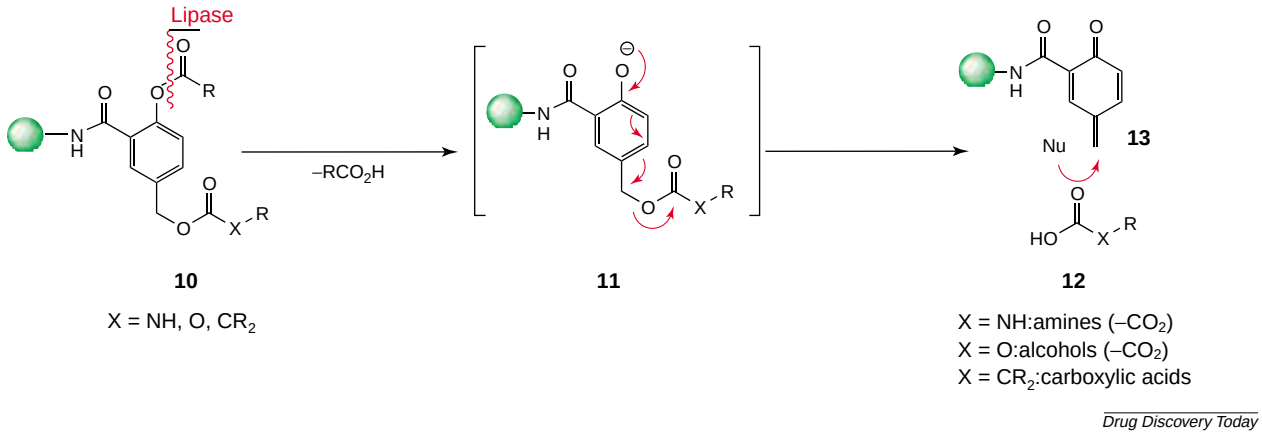

Figure 6. Concept for the development of the enzyme-labile 4-acyloxy-benzyloxy linker group. Red arrows represent enzymatic cleavage.

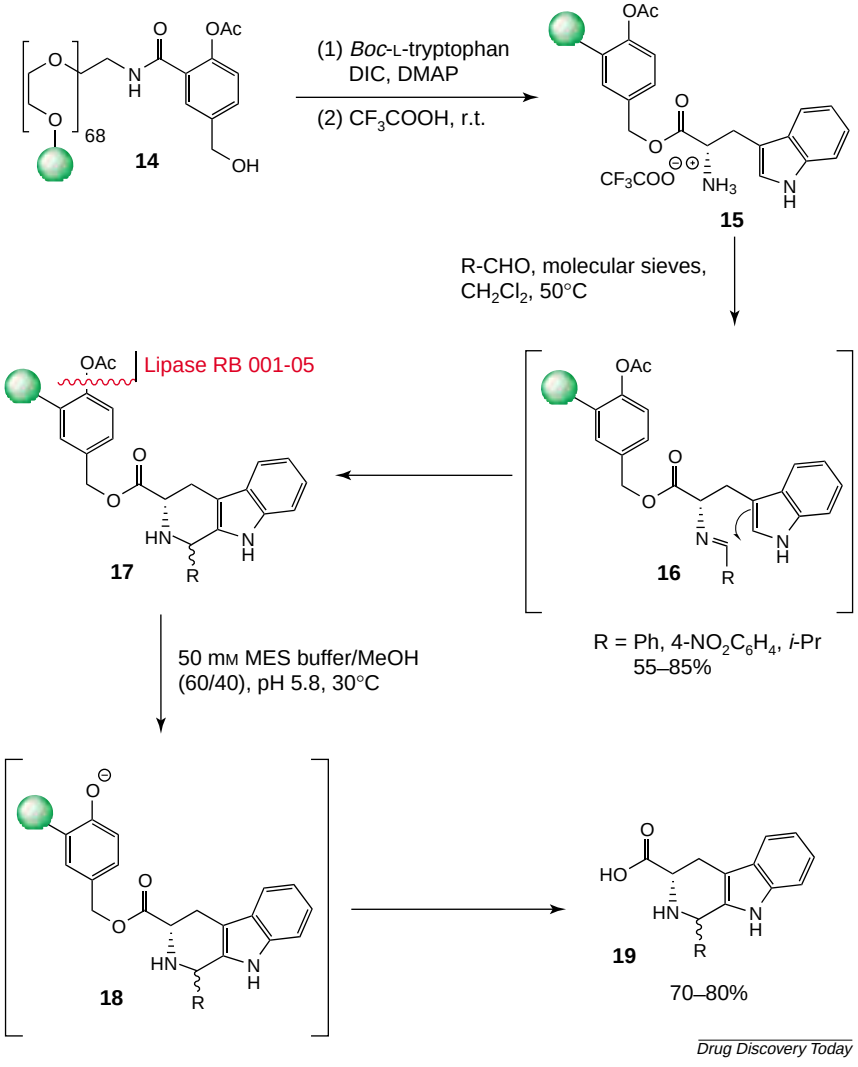

Figure 7. Solid-phase synthesis of tetrahydro-β-carbolins and subsequent detachment by enzyme-initiated fragmentation of the anchor group.

37–60°C, the desired compounds **36–38** (Fig. 10) were isolated by simple extraction with diethyl ether, with yields between 73% and 94% and a purity of >95%. Finally, Mitsunobu esterification products and Diels–Alder reaction products **40** and **43** (Fig. 11) were released from the polymeric support using penicillin G acylase, with high yields and high purity.

Conclusion and outlook

This article has provided an overview of a variety of different approaches involved in the successful development of enzyme-labile linker groups in polymer-supported synthesis. Enzyme-labile linkers fulfill the requirements of a smooth synthetic method for the cleavage of compound libraries from the solid-phase, and can also be used at room temperature and under neutral conditions. We have also shown their applicability to the synthesis of numerous different classes of compounds, including peptides, glycopeptides, oligosaccharides, PNAs, oligonucleotides, natural products, different heterocycles and bicyclic compounds. In addition, these biocatalysts could be promising tools in the synthesis of sensitive and multifunctional compounds that already carry several different protecting groups.

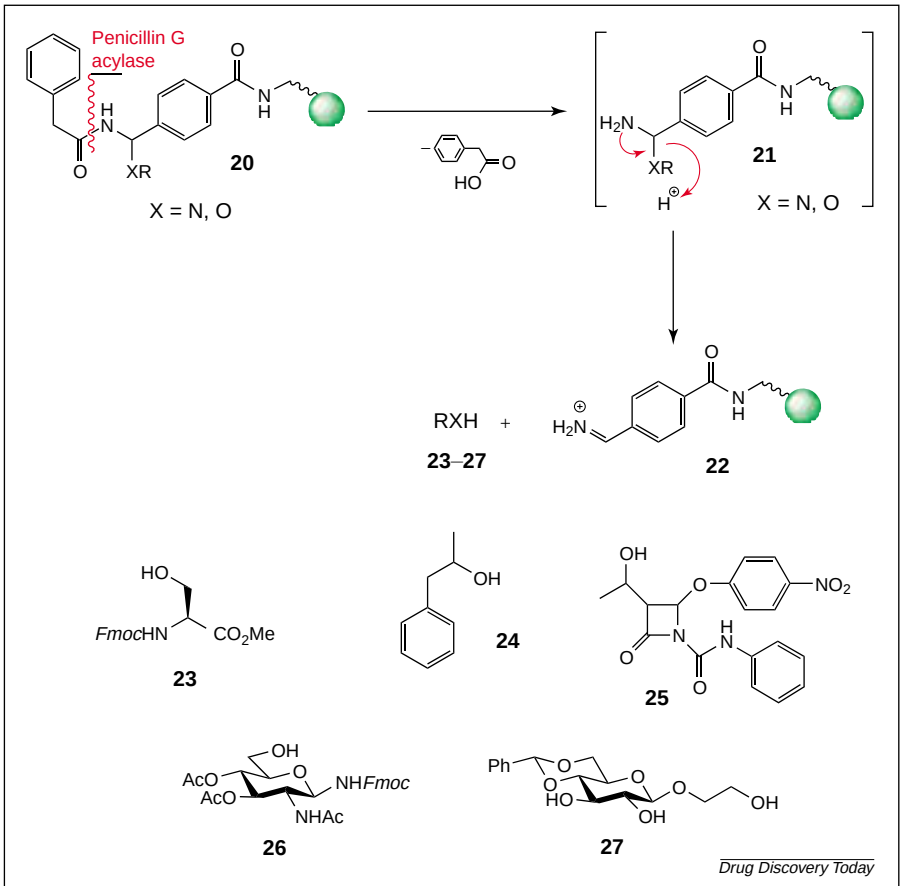

Figure 8. Loading and cleavage of a penicillin acylase scissile linker. Red arrows represent enzymatic cleavage.

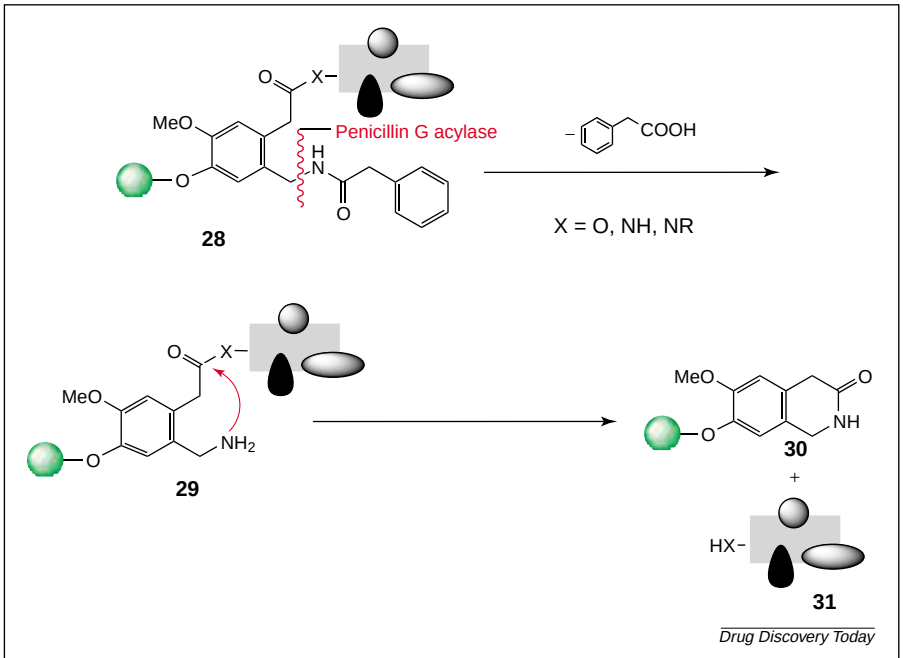

Figure 9. Principle of the enzyme-labile safety-catch linker. The red arrow represents enzymatic cleavage.

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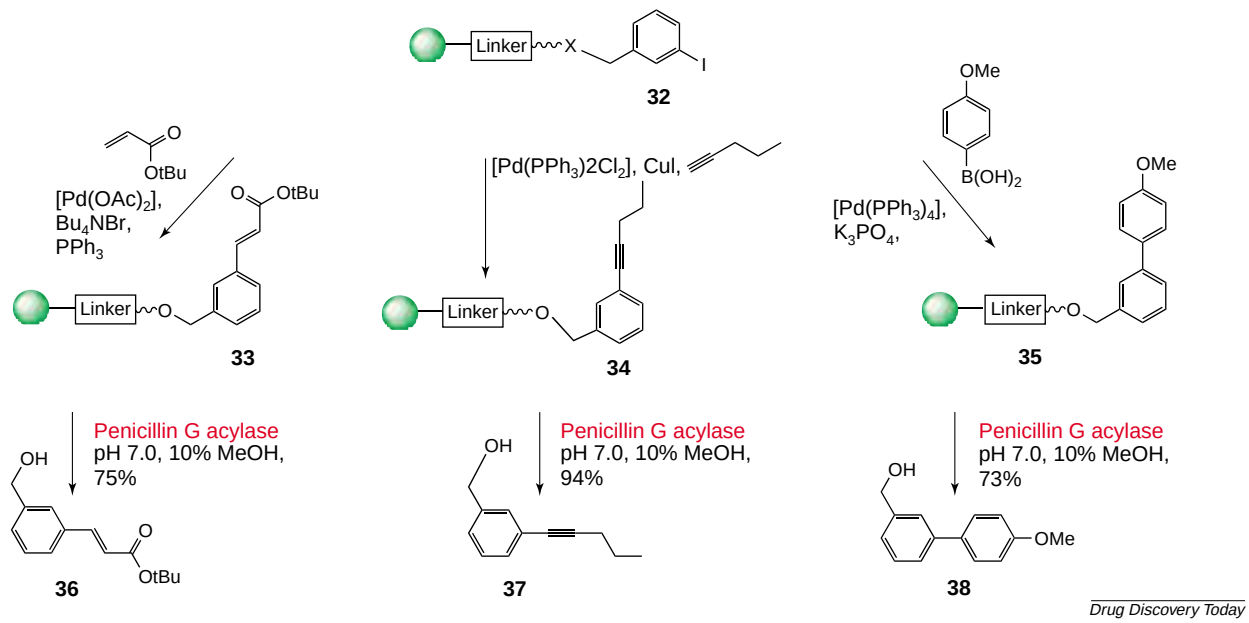

Figure 10. Pd(0)-catalyzed reactions on enzyme-labile linker conjugates.

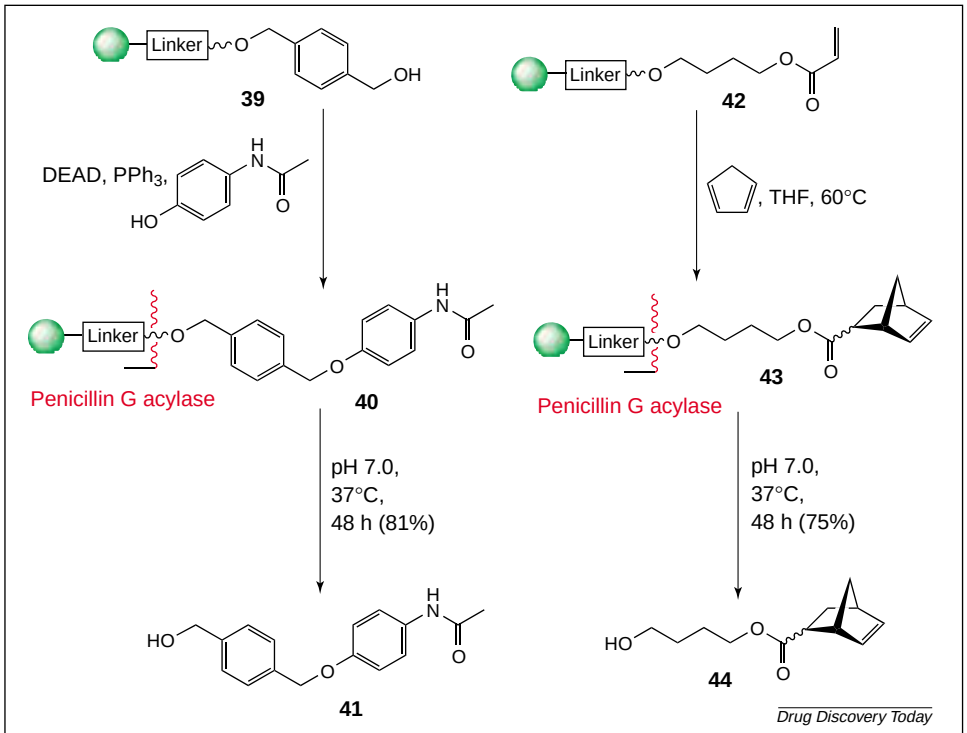

Figure 11. Mitsunobu and Diels–Alder reaction on enzyme-labile linker conjugates.

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