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# Handles for Fmoc Solid-Phase Synthesis of Protected Peptides

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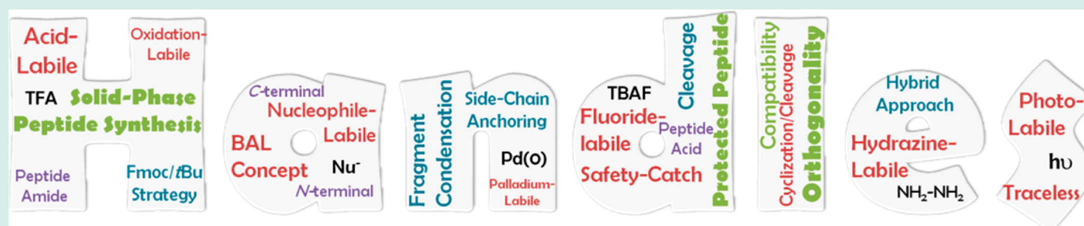


**ABSTRACT:** Protected peptide fragments are valuable building blocks for the assembly of large peptide sequences through fragment condensation approaches, whereas protected peptides are typically synthesized for the preparation of amide-bridge cyclic peptides in solution. Efficient synthesis of both protected peptides and protected peptide fragments by solid-phase peptide synthesis methodology requires handles that attach the growing peptides to the polymeric support and can be cleaved under appropriate conditions, while maintaining intact the side-chain protecting groups. Here, we provide an overview of attachment methods described in the literature for the preparation of protected peptides using Fmoc/*t*Bu chemistry, including the most commonly used acid-labile linkers along with the most recent and sophisticated.

**KEYWORDS:** *handles, solid-phase peptide synthesis, protected peptides, Fmoc/tBu strategy, protecting group orthogonality, fragment condensation approach*

## ■ INTRODUCTION

According to the last Peptide Therapeutics Foundation report,[1] the average frequency of peptides entering clinical studies forty years ago was just over one per year. That number has steadily increased to nearly 20 peptide-based drug candidates entering clinical evaluation annually. Peptide synthesis and discovery therefore remain central to drug development and biomedical research.

The methodology of solid phase peptide synthesis (SPPS), first described by Merrifield in 1963,[2] provided the revolution in the field of peptide chemistry that enabled the introduction of peptides as drugs. Principally, peptide synthesis relies on the appropriate combination of protecting groups and an efficient method for the activation of the carboxyl group prior to reaction with the amino group for growth of the peptide chain in the C-to-N direction. Thus, a general solid-phase peptide synthesis scheme includes a $N^{\alpha}$-amino protecting group (*temporary* protecting group), side-chain protecting groups (*permanent* protecting groups) and a linker,[3] a specialized protecting group that attaches the peptide to the support (Figure 1).

The linker or handle can be regarded as a bifunctional chemical moiety that attaches a compound to a solid or soluble support, is inert under peptide elongation conditions (avoiding premature cleavage during coupling and deprotection cycles), and can be cleaved to release compounds from the support when desired.[4] In general, protecting-group chemistry has been the basis of the development of novel handles and specific cleavage conditions,

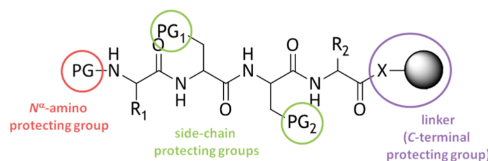


**Figure 1.** Protecting groups for solid-phase peptide synthesis.

enabling the release of the target molecule without degradation and free of byproducts. Linker designs have also expanded from this initial purpose to include novel concepts and functionalities. For instance, many handles have been designed and developed for the preparation of *C*-terminal modified peptides in a variety of ways.[5] Examples include backbone amide attachment (BAL),[6] side-chain anchoring, and inverse solid-phase peptide synthesis, among others. Many are now commonly used synthetic tools incorporated into peptide synthetic schemes. Although acid-labile linkers are by far the most widely used in peptide chemistry, a myriad of handles that can be cleaved by different mechanisms have been described. These include cleavage by base, nucleophiles (thiols, hydrazines, etc.), fluoride, enzymes, light, reducing agents, oxidizing agents, and palladium complexes. The most sophisticated are traceless by virtue of safety-catch or cyclative reactivity.

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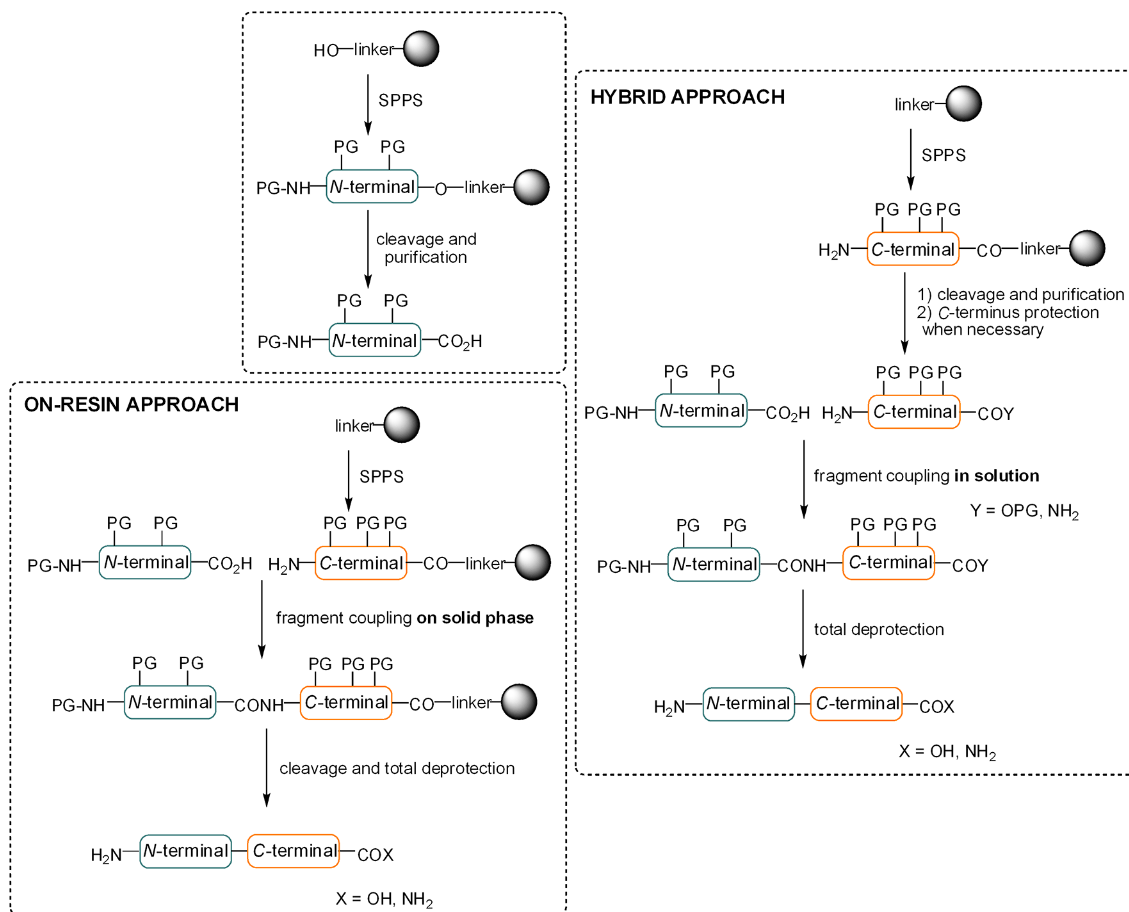
**Scheme 1. Schematic Representation of the Fragment Condensation Approaches for Peptide Synthesis**




Although several handles have been designed and applied for the preparation of modified peptides in a broad concept, we have focused on linkers described for the synthesis of protected peptides on solid phase. Side-chain protection is required for further application in fragment condensation approaches, and the synthesis of protected peptides is often required for the head-to-tail cyclization of peptides in solution. For readers interested in handles used in the preparation of modified peptides by SPPS, several reviews and books have appeared in the past decade.[5,7−9]

## PROTECTED PEPTIDES AND PROTECTED PEPTIDE FRAGMENTS FOR DIVERSE APPLICATIONS

Nowadays, SPPS is a well-established methodology for the synthesis of small- to medium-sized peptides. Originally based on the combination of the Boc/Bzl groups, peptides are now mostly synthesized using the Fmoc/*t*Bu orthogonal strategy.

Because it is a sequential process, the ability to assemble a peptide in a stepwise manner is limited by the synthesis efficiency of each step, including coupling and deprotection. Thus, the synthesis of a 100-residue peptide with 99% efficiency at each step provides a 90% overall yield of the target peptide, while 97% efficiency gives a 5% overall yield.[10] Difficult couplings and deprotection during SPPS are commonly associated with poor solvation of the elongating strand anchored onto the solid support, high tendency of growing hydrophobic peptide sequences to aggregate,[11] or the presence of consecutive hindered amino acids in the sequence.

The development of novel coupling reagents,[12] handles,[2,7−9] and protecting groups,[13] the identification and diminishment of detrimental side reactions, the use of dimethylsulfoxide (DMSO)[14] or "magic mixtures",[15] or the addition of chaotropic salts[16] during coupling/deprotection cycles, along with the emergence of innovative PEG-based resins, such as SPOCC[17] and ChemMatrix,[18,19] have significantly improved the efficiency of the stepwise SPPS approach. Moreover, the use of additional synthetic tools, including the incorporation of pseudoprolines[20−25] or *N*-2-hydroxy-4-methoxybenzyl (Hmb)[26,27] and dicyclopropylmethyl (Dcpm)[28] for backbone protection, as well as the implementation of an *O*-acyl isopeptide method,[29−31] have been successfully applied to increase the overall synthesis efficiency. Despite all these advances, the stepwise SPPS approach is commonly limited to the synthesis of polypeptides shorter than 50 residues because of the accumulation of undesirable byproducts derived from nonquantitative coupling and deprotection reactions during peptide elongation, which lead to truncated or deletion sequences. Convergent approaches are therefore advantageous in many demanding cases.

For instance, fragment or segment condensation strategies, which rely on the preparation of polypeptides by assembling protected peptide fragments (Scheme 1), are commonly adopted for the synthesis of long peptide sequences. The acyl-acceptor-peptide fragments (C-terminal-protected segment) and acyl-donor-peptide fragments (N-terminal-protected segment) are elongated on solid phase, and then, the resulting protected segments are coupled on-resin (solid-phase fragment coupling) or in solution (hybrid strategy), followed by the full removal of the protecting groups. While acyl-donors may be directly used in the fragment coupling reaction in solution, acyl-acceptor fragments

frequently require additional protection transformation of the C-terminal group. The fragment condensation approach requires rapid access to pure protected peptide fragments, which are needed as building blocks. Thus, efficient synthesis of protected N- and C-peptide intermediates requires handles that maintain side-chain protecting groups intact after cleavage.

On the other hand, fully protected peptides are required for the synthesis of amide-bond cyclic peptides in solution. Although on-resin cyclization after peptide assembly on solid phase is preferred, peptide cyclization in solution is implemented when necessary. In this regard, cyclization tendency of linear precursors depends mainly on the size of the ring to be formed and the ring closure of certain hexapeptides or shorter peptide sequences is reported to be hampered. Therefore, side-chain protected peptides are typically cyclized in solution to accomplish the synthesis of small amide-bridge cyclic peptides. In addition, tendency of the linear precursor to cyclize is well-known to be sequence-dependent,[32,33] and in some cases, amide-bond construction is more efficient in solution. Thus, for instance, protected peptides have been implemented for the construction of cyclic peptides for further preparation of a library of protein surface discontinuous epitope mimics[34] or for synthesis of bicyclic homodetic peptide libraries.[35]

Moreover, although different handles have been developed for the preparation of C-terminal-modified peptides on-solid phase, protected peptides may be synthesized for their further C-terminal derivatization in solution. For instance, protected peptides have been recently applied for the preparation of a C-terminal peptide phosphonates library;[36] for the construction of C-terminal modified small Arg-rich peptides as potent furin inhibitors;[37] and for the synthesis of several dye marked diketo-piperazine receptors against an encoded side chain-protected tripeptide library.[38]

## HANDLES FOR SOLID-PHASE SYNTHESIS OF PROTECTED PEPTIDES

Synthesis of protected peptides by SPPS relies on the proper choice of an **orthogonal/compatible** set of protecting groups and linkers. **Orthogonal** protection schemes comprise two or more types of protecting groups which may be removed by different chemical mechanisms, in any order and in the presence of other types of protecting groups. On the other hand, **compatible** protecting groups are those belonging to a similar type of protecting groups cleaved by means of similar chemical mechanisms with different reaction rates, which can be removed in the presence of the other, but just in one order.

We review here a wide range of handles used in the preparation of protected peptides on solid phase, to provide the reader with a practical overview of this topic. Although some of the linkers described here have been reported to be orthogonal and/or compatible with both Boc/Bzl and Fmoc/*t*Bu chemistry, we focus on those linkers described for Fmoc/*t*Bu methods.

**Acid-Labile Handles.** Most acid-labile linkers are based on benzyl, benzhydryl, and trityl systems and are frequently cleaved using trifluoroacetic acid (TFA). Acid lability is related with the stability of the carbocation generated under acidic treatment and can be fairly modulated by incorporating p- or o-electron-donating or -withdrawing substituents from the aromatic rings. Thus, carbocations of trityl structures are more stable than those of benzhydryl moieties, which in turn, are more stable than those derived from benzylic structures. Moreover, the addition of further *p*- or *o*-methoxy groups results in linkers with enhanced acid sensitivities.

**Table 1. Acid-Labile Linkers for Protected Peptide Synthesis on Solid Phase**

| | Name | Structure | Cleavage Conditions | Refs. |
|---|---|---|---|---|
| **1** | SASRIN resin | | 0.5–1% TFA in $CH_2Cl_2$ (peptide acids) | 43 |
| **2** | HMPB linker | | 1% TFA in $CH_2Cl_2$ (peptide acids) | 44,45 |
| **3** | Sheppard linker | | 1% TFA in $CH_2Cl_2$ (peptide acids) | 46 |
| **4** | HAL linker | | 0.1% TFA in $CH_2Cl_2$ (peptide acids) | 47 |
| **5** | Rink acid resin | | 1% TFA in $CH_2Cl_2$ or 10% AcOH in $CH_2Cl_2$ (peptide acids) | 48 |
| **6** | Benzhydryl chloride linker | | 1% TFA in $CH_2Cl_2$ or 25% HFIP in $CH_2Cl_2$ (peptide acids) | 49 |
| **7** | 2-CTC resin | | 1% TFA in $CH_2Cl_2$ AcOH–TFE–$CH_2Cl_2$ (1:2:7) (peptide acids) | 40,50 |
| **8** | Trityl linkers | | 1% TFA in $CH_2Cl_2$ AcOH–TFE–$CH_2Cl_2$ (1:2:7) (peptide acids) | 51 |
| **9** | Trityl linker variants | | AcOH-TFE-$CH_2Cl_2$ (1:1:8) or 0.1% TFA in $CH_2Cl_2$ (peptide acids) | 52 |
| **10** | THAL | | 0.5% TFA in $CH_2Cl_2$ (peptide acids) | 53 |
| **11** | Sieber amide resin | | 1–5% TFA in $CH_2Cl_2$ (peptide amides) | 54 |
| **12** | XAL linkers | | 1–5% TFA in $CH_2Cl_2$ (peptide amides) | 55 |
| **13** | Sieber amide variant | | 1% TFA in $CH_2Cl_2$ (peptide amides) | 56 |
| **14** | Trityl-based | | 1% TFA in $CH_2Cl_2$ (peptide amides) | 57 |
| **15** | Ramage resin | | 3% TFA in $CH_2Cl_2$ (peptide amides) | 58 |

For the synthesis of protected peptide acids, several linkers and resins have been described, including SASRIN resin (**1**), 4-(4-hydroxymethyl-3-methoxyphenoxy)butyric acid (HMPB) linker (**2**), Sheppard linker (**3**), and hypersensitive acid labile (HAL)

linker (**4**), which are based on the benzyl moiety; Rink acid resin (**5**), and a Rink chloride analog (**6**) based on benzhydryl scaffold; along with 2-chlorotrityl chloride (2-CTC) resin (**7**) and various trityl system-based analogs (**8** and **9**). More recently, our group developed linker thiophene acid labile (THAL) (**10**) based on the electron-rich 3,4-ethylenedioxythenyl (EDOT). Among all resins, 2-CTC is the most widely used resin because of its extensive availability and lower cost. In addition, 2-CTC resin offers other synthetic advantages, such as minimization of diketopiperazine (DKP) formation because of the steric hindrance of trityl groups,[39] low epimerization during incorporation of the first amino acid,[40] as well as the possibility of being reused.[41] 2-CTC resin, in addition to allowing the protected fragment to be released by low-acidic mixture of 1−2% TFA in $CH_2Cl_2$, allows cleavage to be accomplished in milder conditions by using trifluoroethanol (TFE)−$CH_2Cl_2$ or hexafluoroisopropanol (HFIP)−$CH_2Cl_2$ mixtures.[42] All these resins and linkers render protected fragment acids with the *C*-terminus unprotected. These protected segments may be directly used in the fragment coupling reaction when they participate as the N-terminal fragment. However, normally, when these fragments correspond to C-terminal intermediates, then the C-terminal carboxylic acid should be previously protected in an additional step before fragment coupling in solution is performed.

On the other hand, general highly acid-labile linkers for the synthesis of protected peptide amides are based on xanthenyl moiety, such as Sieber amide resin (**11**), XAL linkers (**12**), and a Sieber-based analog (**13**), although a trityl-based linker (**14**) has also been described, but poorly used. Moreover, Ramage and coworkers reported the preparation of a linker (**15**) based on the dibenzocyclohepta-1,4-diene moiety. Complete release of peptide amides is achieved with diluted TFA treatments (Table 1).

**Palladium(0)-Labile Handles.** Acid- and base-stable allylic linkers, such as HYCRAM (**16**), *β*-Ala-containing analog thereof (**17**), and HYCRON (**18**) linker, allow peptide release under almost neutral conditions through a palladium(0)-catalyzed allyl transfer to scavenger nucleophiles. Boc-groups, *t*Bu-esters and *t*Bu-ethers remained intact after cleavage treatment (Table 2).

**Fluoride-Labile Handles.** Given the stability of typically used side-chain protecting groups in Fmoc-SPPS chemistry for fluoridolysis, several silicon-based handles have been developed. These linkers enable the release of protected peptides under either basic or neutral conditions. For instance, the so-called "silico Wang" linker Pbs (**19**), developed by Barany's group, demonstrated to yield protected peptides by brief treatments of tetrabutylammonium fluoride (TBAF) in DMF with the presence of appropriate scavengers. The SAC linker (**20**), stable under typical peptide synthesis conditions, permitted to generate protected peptide acids using either fluoride ions or 1% TFA in $CH_2Cl_2$. Other silicon-based handle was described by Ramage and colleagues (**21**). After peptide elongation, cleavage from **21** was performed with TBAF in DMF. Moreover, an interleukin-2 fragment was prepared using a novel silyl linker (**22**), which demonstrated its compatibility with Fmoc-SPPS procedures. This latter linker enabled the release of the protected glycopeptide fragment by treatment with CsF-AcOH. More recently, (2-phenyl-2-trimethylsilyl)ethyl linker (PTMSEL) (**23**) demonstrated to be more sensitive toward fluoridolysis, thus permitting protected peptide release by using TBAF·$3H_2O$ in $CH_2Cl_2$ under almost neutral conditions (Table 3).

**Backbone Acid-Labile (BAL) Handles.** The backbone amide linker (BAL) approach, developed by one of us, relies on the peptide being anchored onto the support through a backbone nitrogen, thus allowing the preparation of C-terminal-modified peptides. The original BAL linker (**24**) was treated with 95% TFA for the proper release of the target peptide, although a later study demonstrated that both original BAL and *o*-BAL (**25**) linkers are surprisingly highly acid-labile. Thus, peptide may be cleaved from the resin by using 1−5% TFA, and therefore can be used for the preparation of protected peptides.

**Table 2. Palladium-Labile Linkers for Protected Peptide Synthesis on Solid Phase**

| | Name | Structure | Cleavage Conditions | Refs. |
|---|---|---|---|---|
| **16** | HYCRAM linker | | Pd(0) + allyl scavenger | 59 |
| **17** | β-HYCRAM linker | | Pd(0) + allyl scavenger | 59 |
| **18** | HYCRON linker | | Pd(0) + allyl scavenger | 60 |

**Table 3. Fluoride-Labile Linkers for Protected Peptide Synthesis on Solid Phase**[a]

| | Name | Structure | Cleavage Conditions | Refs. |
|---|---|---|---|---|
| **19** | Pbs linker | | TBAF-thiophenol-DIEA (1:1.2:0.5) in DMF | 61 |
| **20** | SAC linker | | TFAB (2 equiv) in DMF or TFA-$CH_2Cl_2$ (1:99) | 62 |
| **21** | Ramage linker | | TBAF in DMF | 63 |
| **22** | Nakahara linker | | CsF-AcOH in DMF | 64 |
| **23** | PTMSEL linker | | TBAF·$3H_2O$ in $CH_2Cl_2$ | 65,66 |

[a] TMS = trimethylsilyl.

**Table 4. Backbone Acid-Labile Linkers for Protected Peptide Synthesis on Solid Phase**

| | Name | Structure | Cleavage Conditions | Refs. |
|---|---|---|---|---|
| **24** | BAL linker | | 95% TFA (1-5% TFA) | 67,68 |
| **25** | *o*-BAL linker | | 1-5% TFA in $CH_2Cl_2$ | 68 |
| **26** | NAL-4 linker | | 0.5% TFA in $CH_2Cl_2$ | 69 |
| **27** | T-BAL linker | | 1% TFA in $CH_2Cl_2$ | 70 |

More recently, a series of BAL linkers based on various alkoxynaphthalene core structures, were designed. From this study, the tetraalkoxynaphthalene NAL-4 structure (**26**) was revealed to be highly acid-labile, releasing a *t*Bu-protected dipeptide by treatment with 0.5% TFA in $CH_2Cl_2$. Recently, the same research group designed a novel thiophene backbone amide linker (T-BAL) (**27**) based on the EDOT scaffold which exhibited very high acid-lability (Table 4). Because of the ability of BAL-based handles to prepare C-terminal-modified peptides, these linkers would allow the preparation of protected peptide fragments on solid phase with the C-terminal acid function already protected.

**Safety-Catch Linkers.** Of special interest is the safety-catch linker approach, ideal for its particular activation prior to compound cleavage and release. In this case, the linkage between the C-terminal group and the solid support is stable under SPPS conditions, until the safety-catch system is activated by a specific chemical reaction leading to the release of the compounds. Thus, these linkers require previous activation before product release, thereby allowing employment of conditions that would otherwise cleave the substrate from the solid support (Table 5). Some handles based on this concept have been developed for the release of fully protected peptides. For instance, Pascal et al. reported the use of Dpr(Phoc) safety-catch linker (**28**) for the synthesis of protected segments using Fmoc chemistry. Originally reported for Boc chemistry,[71] the corresponding cyclic acylurea (Imc =2-oxo-imidazolidine-4-carboxylic acid) demonstrated to be stable under typical Fmoc/*t*Bu chemistry procedures and was applied in the preparation of protected peptides. After peptide elongation, release was performed by using NaOH and $CaCl_2$ in iProOH−water to render the protected peptide acid. Peptide release can be also performed by using $NH_3$ to accomplish the corresponding protected peptide amide (Scheme 2).

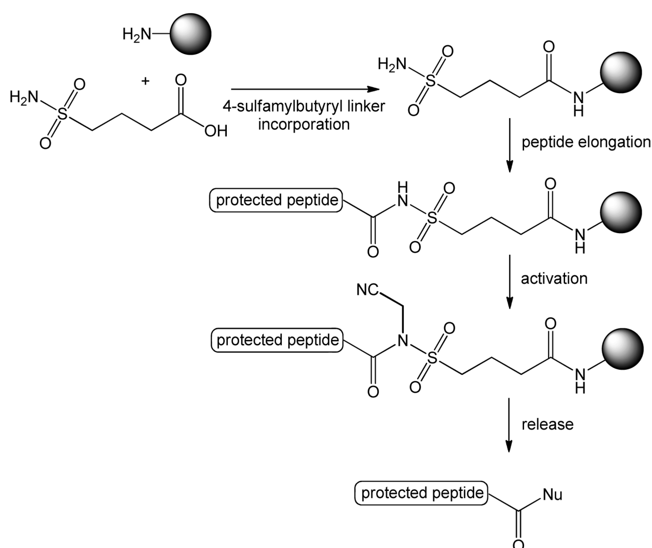
4-Sulfamylbutyryl linker (**29**), also called alkanesulfonamide safety-catch linker, described by Ellman and co-workers, is a variant of the previous Kenner's sulfonamide safety-catch linker.[72] This handle is stable under both basic and strongly nucleophilic reaction conditions, and after treatment with iodoacetonitrile provides *N*-cyanomethyl derivatives that can then be cleaved by nucelophiles under mild reaction conditions to release the target peptide (Scheme 3). This linker has been recently employed in the preparation of a small cyclic peptide.[73]

**Photolabile Handles.** Giese and colleagues designed a photolabile linker based on the 2-pivaloylglycerol moiety (**30**) for the solid-phase synthesis of peptide acids. A protected peptide was successfully cleaved from this linker in high yields

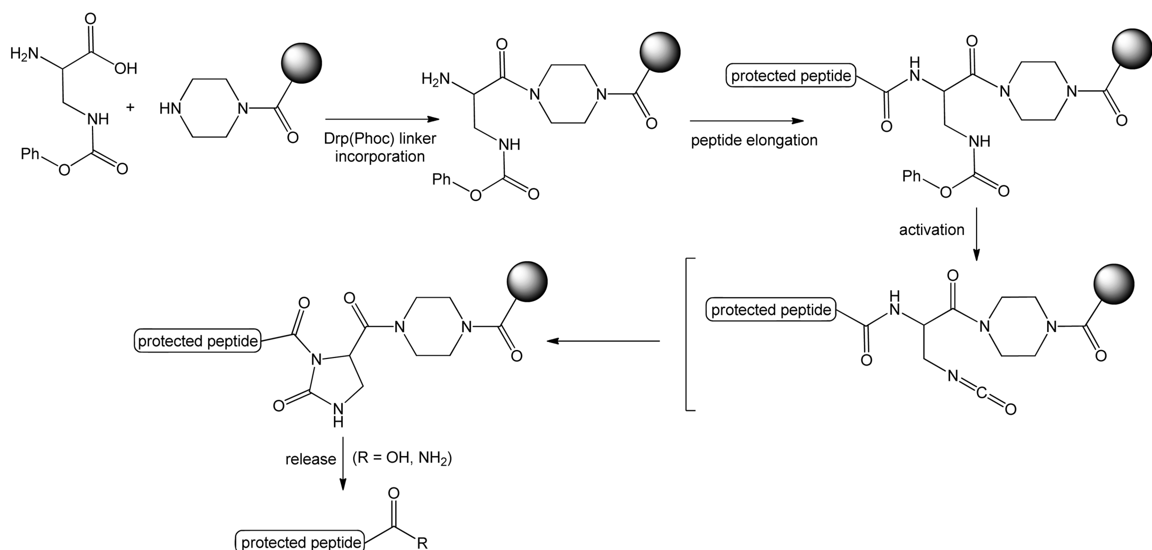
**Table 5. Safety-Catch Linkers for Protected Peptide Synthesis on Solid Phase**

| | Name | Structure | Cleavage conditions | Refs. |
|---|---|---|---|---|
| **28** | Dpr(Phoc) linker | PG–N(H)–CH(COOH)–CH₂–NH–C(O)OPh | activation: PhONa–PhOH<br>release: NaOH, $CaCl_2$ or $NH_3$ | 74,75 |
| **29** | 4-Sulfamylbutyryl linker | $H_2N$–$SO_2$–(CH₂)₃–COOH | activation: iodoacetonitrile-DIEA (20:5) in NMP<br>release:nucleophile | 76 |

**Scheme 3. 4-Sulfamylbutyryl Linker (29) for the Synthesis of Protected Peptides**




**Scheme 2. Dpr(Phoc) Safety-Catch Linker (28) for the Synthesis of Protected Peptides**



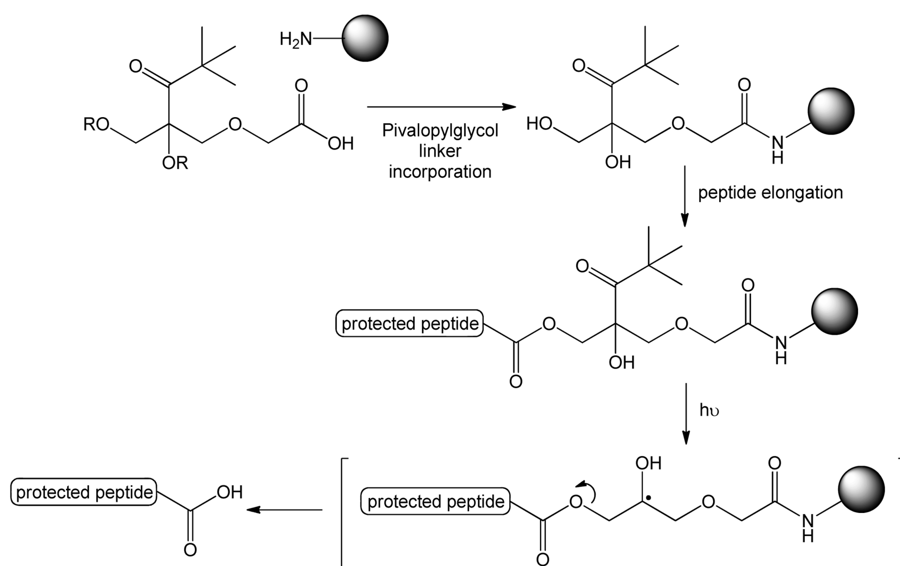
and purities in THF by irradiation at 320−340 nm (Scheme 4). Recently, Copley et al. have designed a photocleavable linker based on the 3′,5′-dimethoxybenzoin moiety, in which the carbonyl group is protected as a dimethyl ketal (**31**) (Scheme 5). This handle is compatible with the Fmoc/*t*Bu strategy and can be quantitatively removed by 3% TFA in $CH_2Cl_2$ followed by irradiation at 350 nm in aqueous or partially aqueous media (Table 6).

**Miscellaneous Handles.** (Azidomethyl)benzamide linker (**32**) was developed for use in solid-phase peptide synthesis, which allows a protected peptide to be cleaved from the resin under neutral conditions by using $Bu_3P$ in a mixture DMF-imidazole buffer at pH 7, while maintaining the *t*Bu, Boc and Fmoc protecting groups intact (Scheme 6). The aryl hydrazine linker (**33**) is a versatile handle which allows the preparation of a wide variety of *C*-terminal-modified peptides. This linker is stable

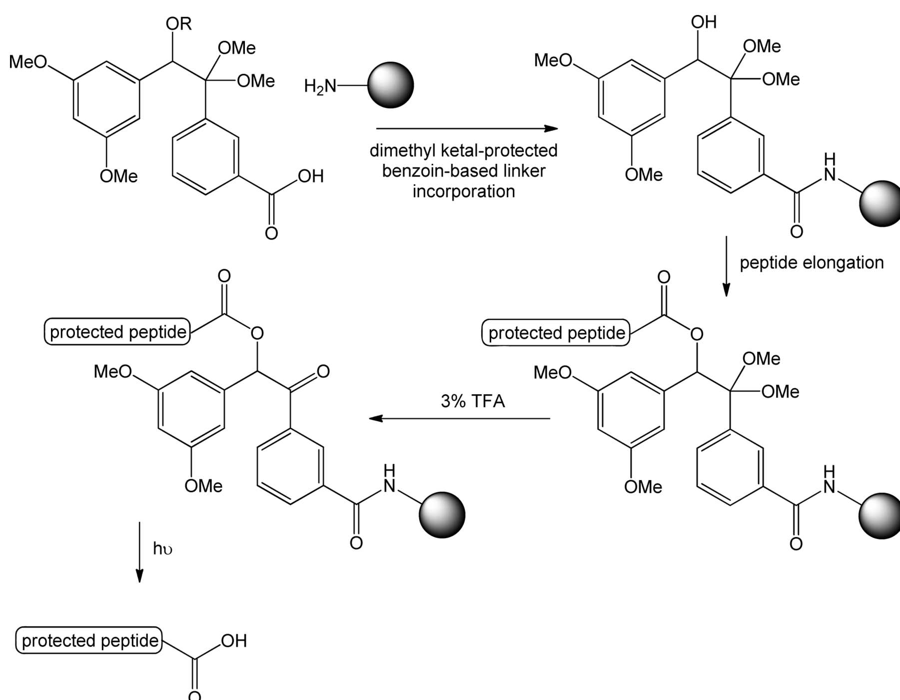
**Table 6. Photolabile Linkers for Protected Peptide Synthesis on Solid Phase**

| | Name | Structure | Cleavage Conditions | Refs. |
|---|---|---|---|---|
| **30** | Pivalopylglycol linker | | hυ at 320-340 nm | 77 |
| **31** | Dimethyl ketal-protected benzoin-based linker | | 3% TFA and hυ at 350 nm | 78 |

**Scheme 4. Photolabile Pivalopylglycol Linker (30) for the Synthesis of Protected Peptides**




**Scheme 5. Photolabile Dimethyl Ketal-Protected Benzoin-Based Linker (31) for the Synthesis of Protected Peptides**





dx.doi.org/10.1021/co300153c | ACS Comb. Sci. 2013, 15, 217−228

to acids and bases and is cleaved with high specificity under mild oxidative conditions in the presence of nucleophiles and basic media, yielding a broad range of C-terminal functionalities. The hydrazine linker is oxidized to acyldiazene by either $O_2$ in the presence of Cu(II) salts and a nucleophile, or by using *N*-bromo-succinimide (NBS), followed by a nucleophilic cleavage. This latter procedure is preferred when oxidative-sensitive residues such as Trp, Tyr and Cys are contained in the peptide sequence (Scheme 7). A selenyl liker (**34**) was designed for the solid-phase synthesis of protected peptides which contain a dehydro-alanine residue. From the selenyl linker, growth of the peptide sequence can be performed in either the C-to-N or the N-to-C direction. Thus, after peptide elongation by using Fmoc/*t*Bu procedure, an oxidative cleavage by treatment with $H_2O_2$ in THF led to the fully protected dehydroAla-containing peptide (Scheme 8). Dde-based linker (**35**) is stable under standard basic conditions employed in the Fmoc/*t*Bu strategy, and can be easily cleaved in 2% hydrazine monohydrate. This linker was successfully applied in the synthesis of side-chain protected and unprotected peptides (Scheme 9). More recently, a triazene function was used to anchor a Phe residue onto a solid support through its side chain (**36**) (Scheme 10). This versatile triazene linkage allowed the preparation of Phe-containing cyclic, *C*-modified, and protected peptides by using 2−5% TFA in $CH_2Cl_2$ and successive reduction of the resulting diazonium salt by using $FeSO_4 \cdot 7H_2O$ in DMF for the release of the target products (Table 7).

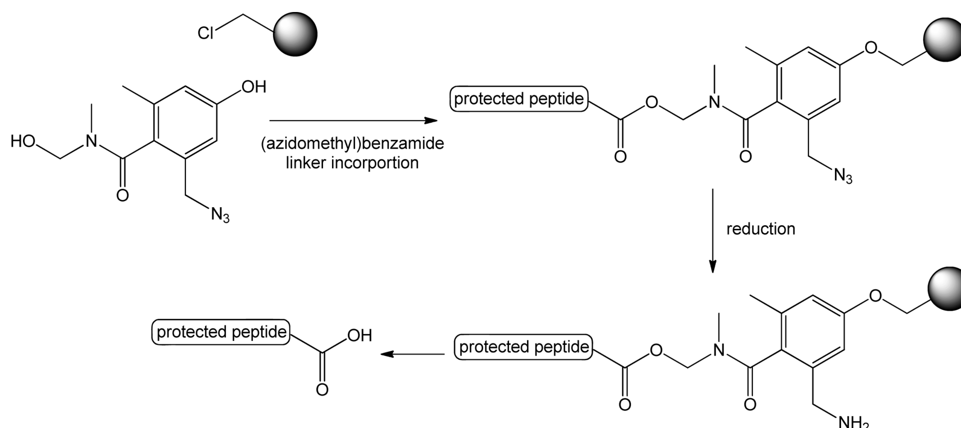
Cyclative cleavage, also so-called cyclorelease strategy, relies on the release of a compound generated by an intramolecular nucleophilic displacement at the linker, which in turn results in a cyclized product. Following this approach, our group has recently described a novel handle for the preparation of fully protected peptides based on the formation of a DKP moiety (**37**) (Table 7).

**Table 7. Several Linkers for Protected Peptide Synthesis on Solid Phase**

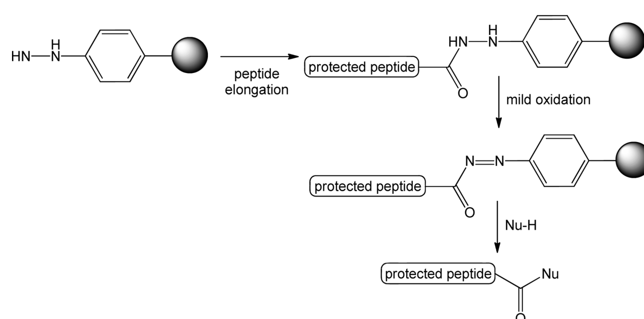
| | Name | Structure | Cleavage Conditions | Refs. |
|---|---|---|---|---|
| **32** | (Azidomethyl) benzamide linker | | $Bu_3P$ in DMF–imidazole buffer at pH 7 | 79 |
| **33** | Aryl hydrazine linker | | $O_2$ + Cu(II) salts + $Nu^-$ or NBS + $Nu^-$ | 80,81 |
| **34** | Seleno-based linker | | $H_2O_2$ in THF (dehydroAla-containing peptides) | 82 |
| **35** | Dde-based carboxy linker | | 2% v/v $NH_2$-$NH_2$ | 83 |
| **36** | Phe-triazene linker | | 2-5% TFA + $FeSO_4 \cdot 7H_2O$ | 84 |
| **37** | DKP-based handle | | PG removal and basic treatments (*C*-terminal protected) | 85 |

First, a dipeptidyl moiety is incorporated into hydroxymethyl resin, followed by the assembly of a bifunctional linker. Next,

**Scheme 6. (Azidomethyl)benzamide Linker (32) for the Synthesis of Protected Peptides**




**Scheme 7. Aryl Hydrazine Linker (33) for the Synthesis of Protected Peptides**





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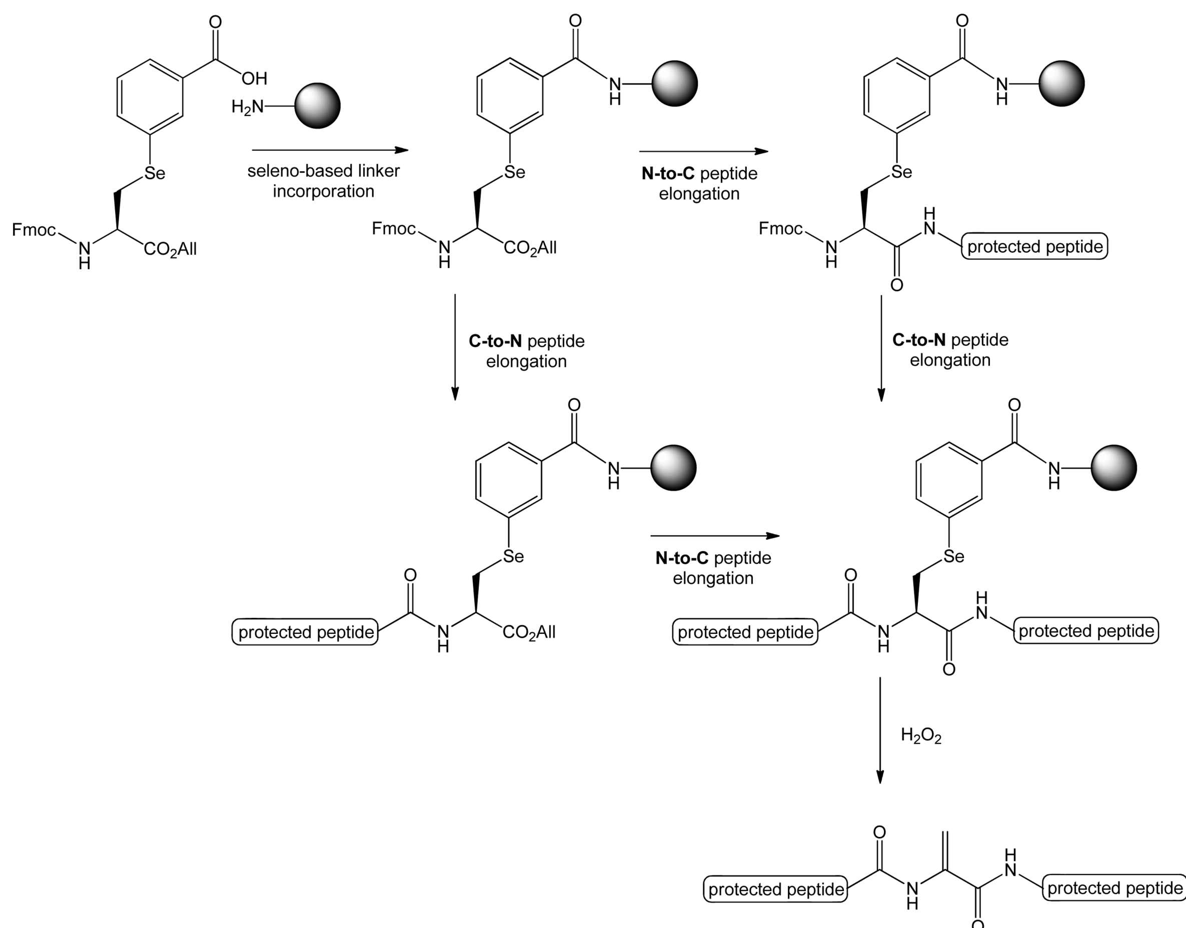
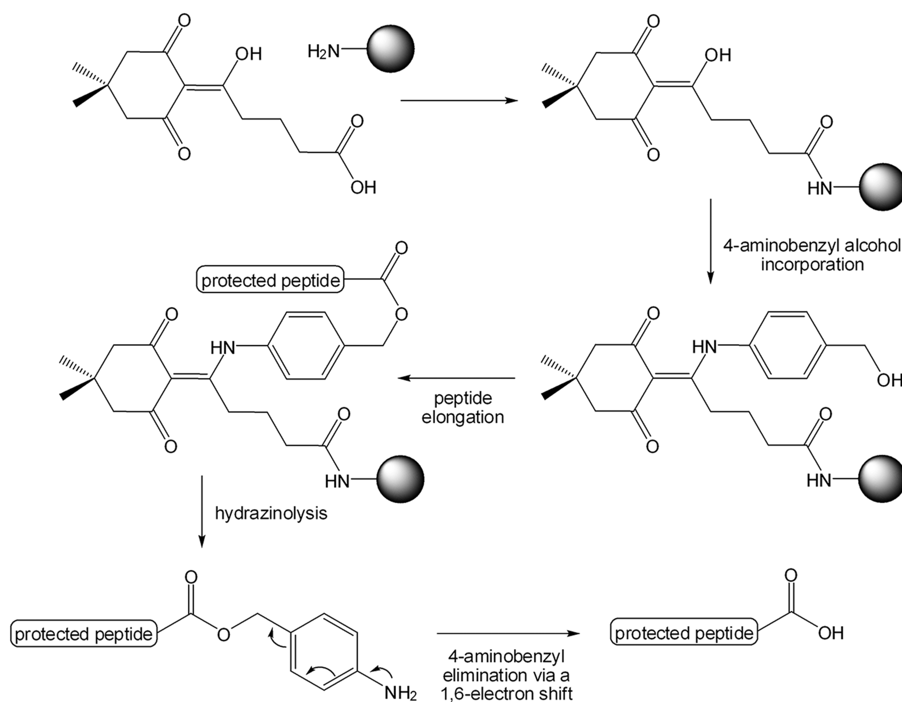
**Scheme 8. Seleno-Based Handle (34) for the Preparation of Dehydroalanine-Containing Protected Peptides**




**Scheme 9. Dde-Based Carboxy Linker (35) for the Preparation of Protected Peptides**




after peptide elongation by Fmoc-SPPS, the $N^{\alpha}$-amino protecting group (PG) comprised on the dipeptidtyl moiety is selectively removed and then treatment with piperidine or pyrrolidine promotes DKP formation, intramolecular cyclization, and concomitant release



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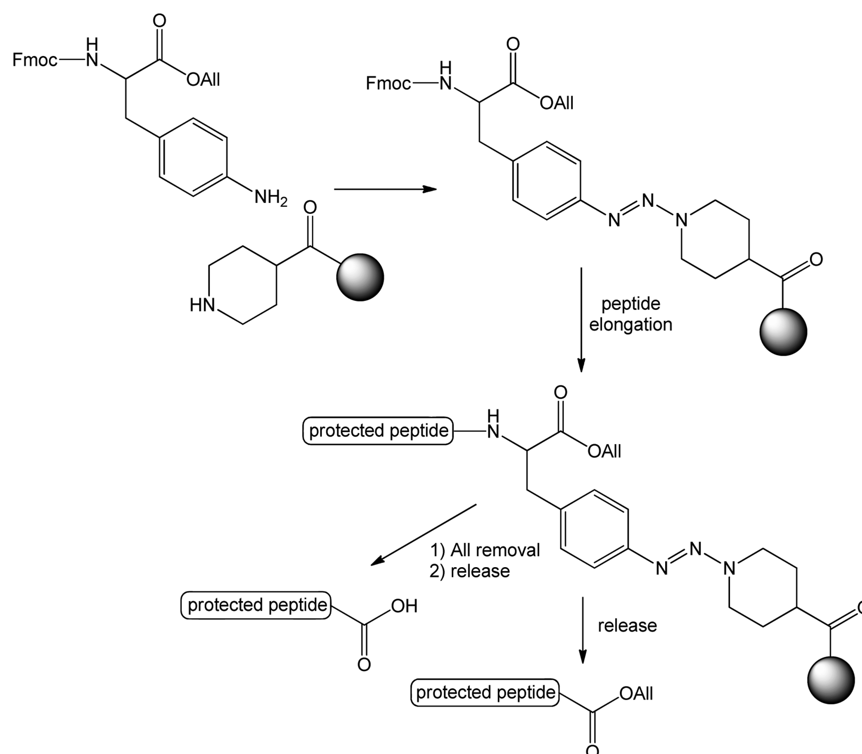
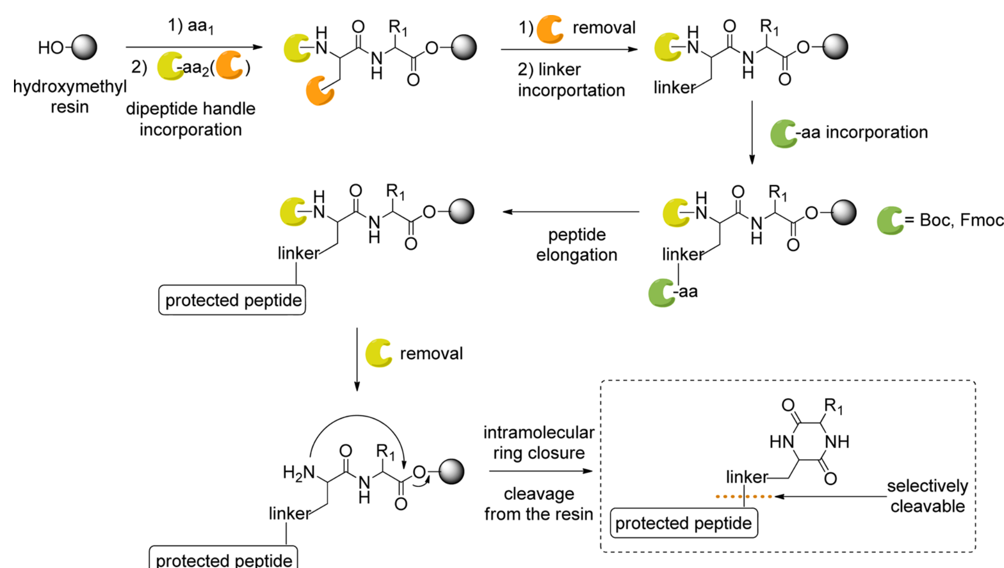
**Scheme 10. Phe-Triazene Linker (36) for the Synthesis of Protected Peptides**




**Scheme 11. Schematic Representation of the DKP-Based Handle (37) Strategy for the Preparation of C-Terminal-Protected Peptides**




of the fully protected peptide. Singularly, the DKP moiety remains as a C-terminal protecting group which can be selectively removed when necessary. This versatile handle is designed so that it can incorporate any bifunctional linker between the dipeptidyl moiety and the target peptide, thereby allowing further selective deprotection of the C-terminal DKP-moiety under specific conditions (Scheme 11).

## ■ OUTLOOK

In conclusion, this work has demonstrated that chemists are continuously developing strategies for the preparation of modified peptides to fulfill the growing needs of researchers working in a broad range of scientific areas in which peptides are important cornerstones. Orthogonal combinations of Fmoc/*t*Bu strategy with diluted-acid-labile, palladium-labile, silicon-based, BAL approach, photolabile, and safety-catch linkers, among others, have been designed and developed for the synthesis of protected peptides for their further application in the fragment condensation approach. A further step has been achieved with the DKP-based handle (**37**), which allows the preparation of C-terminal protected fragment by SPPS that can be used directly for fragment coupling in solution. Although highly acid-labile handles are frequently employed for this purpose, many innovative linkers have been described and successfully applied, thus expanding the range of available synthetic tools for SPPS.



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### Notes

The authors declare no competing financial interest.

## ■ ABBREVIATIONS

BAL, backbone amide attachment; Boc, *tert*-butoxycarbonyl; *t*Bu, *tert*-butyl; Bzl, benzyl; 2-CTC, 2-chlorotrityl chloride; DIEA, *N,N*-diisopropylethylamine; DMF, *N,N*-dimethylformamide; DMSO, dimethylsulfoxide; DKP, diketopiperazine; EDOT, 3,4-ethylenedioxythenyl; Fmoc, 9-fluorenylmethyloxycarbonyl; HAL, hypersensitive acid labile; HFIP, hexafluoroisopropanol; 4-(4-Hydroxymethyl-3-methoxyphenoxy)butyric acid; NAL-4, tetraalkoxynaphthalene; NBS, *N*-bromosuccinimide; NMP, *N*-methylpyrrolidone; PEG, polyethylenglycol; PG, protecting group; RP-HPLC, reverse-phase high-performance liquid chromatography; SAC, silyl acid linker; SARSIN, super acid sensitive resin; SPPS, solid-phase peptide synthesis; TBAF, tetrabutylammonium fluoride; T-BAL, thiophene backbone amide linker; TFA, trifluoroacetic acid; TFE, trifluoroethanol; THAL, thiophene acid labile; THF, tetrahydrofuran; XAL, xanthenyl

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## NOTE ADDED AFTER ASAP PUBLICATION

This paper was published to the Web on April 26, 2011, with errors to Scheme 1, Scheme 9, Structure 35 from Table 7, and the reference section. The corrected version reposted May 2, 2013.