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Photolabile Linkers for Solid-Phase Synthesis

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ABSTRACT. Photolabile linkers are the subjects of intense research, because they allow the release of the target molecule simply by irradiation. Photochemical release of synthesis products is often facilitated without additional reagents under mild reaction conditions, which may even be environmentally friendly and appealing in the context of greener chemistry. The mild conditions also allow for applications of released material in subsequent biological screening experiments, where contamination with cleavage reagents would be detrimental. This review pays attention to the increasing number of photolabile linkers developed for solid-phase synthesis and release, and covers: (i) o-nitrobenzyloxy linkers, (ii) o-nitrobenzylamino linkers,
(iii) α-substituted o-nitrobenzyl linkers, (iv) o-nitroveratryl linkers, (v) phenacyl linkers, (vi) p-
alkoxyphenacyl linkers, (vii) benzoin linkers, (viii) pivaloyl linkers, (ix) other photolabile
linkers.

INTRODUCTION

Originally pioneered by Merrifield for the synthesis of peptides,[1] solid-phase organic
synthesis (SPOS) is a well-established technique that offers unique advantages over conventional
solution-phase methods. With experimental simplicity, reactions are driven to completion with
excessive amounts of reagent, and even highly polar and polymeric organic compounds are
readily handled through multiple reaction steps, and easily isolated following release of product
from the solid support. Since the introduction of solid-phase methods half a century ago, many
laboratories have focused on the development of technologies and chemistry suitable for solid-
phase synthesis, which has resulted in a remarkable outburst of chemical transformations that can
be applied for the routine synthesis of organic molecules on solid support.[2, 3, 4, 5, 6] This has
led to the application of solid-phase synthesis for the generation of molecular libraries in
academia and industry to identify new bio-active molecules, drugs, catalysts and other fine
chemicals.[7, 8] Solid-phase synthesis is the cornerstone of combinatorial chemistry, either
through the generation of nucleic acid libraries for biological display, or in in-bead based split-
pool approaches.

The use of solid supports in organic synthesis relies on several interconnected
requirements. The substrate containing multiple chemical handles must be attached to the solid
support, and then modified through multiple chemical transformations before controlled release
of the target product. Conventional linkers normally used in SPOS typically rely on harsh cleavage conditions, comprising strong acids, bases or nucleophiles, which can pose compatibility problems with more sensitive molecular structures. Inherently, the range of chemical transformations available for the synthesis of any given compound is restricted by linker. Photolysis offers a method of linker cleavage, which is fully orthogonal to conventional chemical methods and therefore, holds a unique promise for applying an expanded range of chemical transformations to build structurally diverse compounds on solid support. Photolabile linkers generally do not need acidic, basic or metal-assisted activation for cleavage, and photochemical substrate release is often facilitated without additional reagents under mild reaction conditions, that may even be environmentally friendly and appealing in the context of greener chemistry. The mild conditions may, furthermore, become attractive for applications of released crude material in subsequent biological screening experiments, where contamination with cleavage reagents would be detrimental. Recent advances in LED lighting technology have significantly increased the ease with which photochemical transformations may be achieved, and the field has witnessed a markedly increased interest in recent years. Herein, we provide an extensive overview of the synthesis and applications of photolabile linkers for solid-phase synthesis.

PHOTOLABILE LINKERS

o-Nitrobenzyloxy linkers

Nitrobenzyl-based linkers are often considered the most commonly used linkers. Their cleavage mechanism is traditionally identified as a Norrish type II reaction, as the photochemistry was described by Norrish in 1935.[9] Norrish demonstrated that an incident
photon (300-350 nm) breaks the N=O π-bond in the nitro-group, forming a diradical excited state. During the photoisomerization, the activated oxygen of the nitro group abstracts a proton from the benzylic position, forming the aci-nitro compound, which subsequently rearranges to afford an o-nitrosobenzaldehyde, thereby releasing the product (Figure 1).

![Figure 1. Norrish Type II mechanism for the photo cleavage of a 2-nitrobenzyl-based linker.](image)

Overall, nitrobenzyl-based linkers are highly general, and the list of functional groups that can be released are now extensive. The scope includes, but is not limited to phosphates, carboxylates, carbonates, carbamates, and alkoxides. Research efforts towards raising the chemical and quantum yields of nitrobenzyl-based linkers have led to the identification of several beneficial modifications, which will be discussed in the following sections.

The first reported example of a photolabile linker was the o-nitrobenzyloxy based resin pioneered by Rich and Gurwara in 1973.[10] The resin was constructed using a Friedel-Crafts-type chloromethylation of the aromatic rings of polymer 1, cf. Scheme 1. A nitration, as described by Merrifield,[1] made the resin ready for attachment of the first amino acid at the C-terminus affording 3. After standard SPPS, photolysis released the first peptide constructed on a photolabile linker in a yield of 62%.
Scheme 1. First solid-phase synthesis and application of a photolabile linker based on the o-nitrobenzyloxy functional group.

However, it became clear that this strategy was not applicable to the synthesis of peptides incorporating more than four amino acids.[11] Over-nitration of the resin apparently caused enhanced polarity of the resin resulting in reduced swelling in organic solvents. In an attempt to find a solution to this limitation, a new strategy was developed where the linker 8 was prepared separately and then coupled to the resin,[11] cf. Scheme 2. Construct 9 was then set up for attachment of the first amino acid and following standard SPPS, the decapeptide 11 was released in 64% yield.
Scheme 2. Synthesis and application of an o-nitrobenzyloxy linker.

Later, Merrifield and coworkers have used linker 8 for the preparation of multi-detachable resins [12, 13]. Barany and Albericio have applied the linker in an orthogonal protection group scheme for solid-phase organic synthesis [14], where the photolabile construct 12 contained a phenolic t-butyl group and a dithiasuccinoyl N-α amino protecting group, both of which are photochemically inert and mutually orthogonal (Scheme 3).

Scheme 3. Three-dimensional and orthogonal protection of a resin-bound peptide.
Different variations of this linker construct have been explored, e.g. with a phenyl group in the benzylic position,[15, 16] or a glycine unit as an internal standard.[17] Pillai et al. has also reported use of the o-nitrobenzyloxy linker with a PEG spacer in between the photolabile cleavage site and the solid support.[18] In a study aiming for the synthesis of encoded small molecule libraries, Ohlmeyer and coworkers used the o-nitrobenzyloxy linker to facilitate easy release of the synthesized compounds under conditions optimal for direct use in biological assays.[19]

In 1993 Greenberg and coworkers demonstrated the application of o-nitrobenzyloxy-based linkers for oligonucleotide synthesis.[20, 21, 22] A DCC-mediated coupling of succinatothymidine 13 to the trichlorophenyl ester 14, followed by coupling to a long-chain alkyl amine controlled pore glass, LCAA-CPG, support in the presence of HOBT led to construct 15, which allowed for the synthesis of oligonucleotides (Scheme 4).

![Scheme 4](image)

**Scheme 4.** Solid-phase oligonucleotide synthesis using a photolabile linker.

Alcohol-containing groups can also be attached to a o-nitrobenzyloxy linker as either carbonates,[23] or ethers,[24] for example in oligosaccharide synthesis. In pioneering work by Zehavi et al.,[25] however, a rather low isolated yield of oligosaccharides was obtained (e.g. 0.87 % of 16, Scheme 5). The low yield was ascribed to low accessibility of amine handles in the
aminoethyl substituted polyacrylamide gel resin used. Later, Nicolaou et al.,[26, 27] Kantchev and Parquette[28] demonstrated the use of the \( o \)-nitrobenzyloxy linker in oligosaccharide synthesis, allowing for a higher isolated yield of the desired oligosaccharides.

**Scheme 5.** Oligosaccharide synthesis utilizing a photolabile \( o \)-nitrobenzyloxy linker.

Using the linker construct 14, amines can be attached through a carbamate moiety as shown by Armstrong et al. for combinatorial library synthesis using the Passerini reaction (Scheme 6).[29]
Scheme 6. Photolabile o-nitrobenzyloxy linker attached to an amine through a carbamate moiety.

2-(2-Nitrophenyl)propyloxy carbonyl (NPPOC) linker

To lower the photolysis time required for efficient cleavage compared to o-nitrobenzyl linkers, Damha et al. hypothesized that extending the chain containing the reactive hydroxyl handle by one carbon atom and branching it to generate a tertiary benzylic carbon center would be beneficial.[30] The effects of a substituent in the benzylic position are consistent with the relative rates of hydrogen abstraction from alkyl carbon by a photo-activated o-nitro group: primary < secondary < tertiary.
To investigate this feature, Damha et al. prepared linker 28.[30] Starting from 26, linker 28 was synthesized in two steps followed by attachment to the solid support (Scheme 7). With construct 29 in hand, Damha synthesized a library of oligoribonucleotides and found that the target molecules indeed were photolytically released from the linker in only 15 minutes at room temperature.

Scheme 7. Photolabile o-nitrobenzyloxy linker for incorporation of a tertiary benzylic carbon.

Despite the structural similarity between o-nitrobenzyl linkers and 28, the release mechanism differs markedly. The generated aci-nitro intermediate rearranges to o-nitrostyrene with β-elimination of the linker group,[31] as illustrated in Figure 2.
**Figure 2.** Mechanism for the photocleavage of NPPOC derivatives.

**o-Nitrobenzylamino linkers**

Taking into account that many biologically active peptides possess a c-terminal primary amide, Rich and Gurwara developed a variation of the o-nitrobenzyl-based linker that upon photolysis releases amides (Scheme 8).[32] Starting from p-toluic acid, bromination followed by nitration led to 8. The bromide 8 was then aminated and immediately Boc-protected using Boc-azide to furnish 32. After attachment to the resin and deprotection, Boc-Val-OH was attached. After photolysis Boc-Val-NH$_2$ (35) was provided in quantitative yield. Furthermore, it was shown that a decapeptide could be prepared and released in high purity and good yield (65%). Another synthetic route to provide 32 from 4-aminomethylbenzoic acid was presented by Barany and coworkers.[33] Ajayaghosh and Pillai demonstrated the possibility of releasing secondary amides by substituting the linker precursor 8 (or the corresponding chloride) with a primary amine instead of ammonia.[34, 35, 36] Hinterstein, *et al.* used an o-nitrobenzylamino linker in their on-bead screening of a one-bead one-compound library in the search for nuclear import inhibitors.[37]
Scheme 8. Photolabile o-nitrobenzylamino linker for release of amides.

Recently, Seeberger and coworkers developed an approach for the automated synthesis of glucosaminoglycans utilizing an o-nitrobenzylamino linker.[38] Iterative cycles of coupling carbohydrate building blocks 37 and 38 to the linker construct 36, yielded, in 16 steps, the hexasaccharide 40 in 13% yield after photocleavage in a continuous flow photoreactor, cf. scheme 9.

Later, the same linker construct (36) was used for the synthesis of a 30-mer mannoside [39] and for the synthesis of $\beta$-(1,3)-glucans [40].
**α-Substituted o-nitrobenzyl linkers**

The o-nitrobenzyl-based linkers presented so far suffer from disadvantageous formation of side products, such as o-nitrosobenzaldehyde, which is a very reactive species prone to polymerization, forming a highly colored product, which may act as an undesired internal light filter that affects the efficiency of the photolytic release.[41] To solve this issue, Ajayaghosh and Pillai prepared resin 43, featuring a secondary benzyl alcohol as reactive handle for peptide attachment (Scheme 10).[41]

![Chemical structure](image)

**Scheme 10.** Integral α-substituted nitrobenzyl-derived photolabile linker used for the synthesis of pentapeptide 46.

Resin 1 was subjected to Friedel-Crafts-type acylation, followed by reduction to the alcohol-functionalized resin 42, which after substitution and nitration was transformed into the bromo-functionalized integral linker 43. This linker was used for the synthesis of pentapeptide 46 in a yield of 40%. This resin, however, suffered from problems similar to those observed with Rich
and Gurwara’s original \( o \)-nitrobenzyloxy linker (Scheme 1). Over-nitration destroyed the swelling properties of the resin and prevented the synthesis of longer peptides.

A solution to this issue is to introduce the support itself in the benzylic position instead of a methyl group (Scheme 11).[42] The resin 1 underwent Friedel-Crafts acylation followed by reduction leading to \( o \)-nitrobenzhydryl resin 48. With this resin in hand, Ajayaghosh and Pillai synthesized peptides 49[42] and, after transformation to the amine-functionalized resin 50,[43] the corresponding amidopeptides 51. Peptides with up to 10 amino acids were synthesized in good yields.

![Scheme 11. \( o \)-Nitrobenzhydryl resin for the synthesis of peptides and amidopeptide.](image)

The related 3-amino-3-(2-nitrophenyl)propionyl (ANP) linker 53 was developed by Geysen and coworkers for the synthesis of peptide amides (Scheme 12).[44] Sternson and Schreiber demonstrated a more acid-stable variant established with two methyl groups in the \( \alpha \)-position of the carboxylic acid handle.[45] Geysen and coworkers demonstrated the use of an alcohol-functionalized variant of the linker for oligosaccharide synthesis.[46] A longer spacer was incorporated between the support and the photosensitive part to avoid problems with \( \beta \)-elimination and lactonization of the alcohol.
Scheme 12. Synthesis of photolabile ANP resin used for the synthesis of amidopeptide 55.

An alternate route to similar linkers was designed by Ryba and Harran to access hydroxyl (60) and amino-containing variants (61), respectively, both from the ketone 56 (Scheme 13).[47]

Scheme 13. Synthesis of photolabile ANP type linkers.

**o-Nitroveratryl linkers**

First introduced by Zehavi and Patchornik,[48] the use of the o-nitroveratryl group (4,5-dimethoxy-2-nitrobenzyl) in photolabile linkers was popularized in the mid-1990s by Yoo and Greenberg,[49] and Holmes and Jones.[50]

Yoo and Greenberg utilized the linker construct to circumvent some of the issues encountered with the original, unsubstituted o-nitrobenzyl linker, namely long photolysis times and low yields...
of release.[48] The synthesis of linker 64 started from vanillin (62), which after etherification and nitration provided the aldehyde 63, cf. Scheme 14. After a few functional group interconversions, linker 64 was obtained.[48]

**Scheme 14.** Synthesis of photolabile o-nitroveratryl-based linker.

Linker 64 can readily be installed on a LCAA-CPG support as construct 67, which was used for the synthesis and release of oligonucleotides in good yields (Scheme 15).[48] Later, it was shown that a more efficient photolysis was achieved when the hydroxyl group of the nucleotide was attached directly to the linker via a carbonate group.[51] Initiated by these studies, this linker has been more broadly implemented in oligonucleotide synthesis.[52]
Holmes and Jones introduced an o-nitroveratryl-based amine linker with a methyl group in the α-position.\[49\] This linker, producing amides upon photolysis, also solved the problem relating to formation of reactive nitroso aldehydes upon photolysis, as described earlier. The synthesis from acetovanillone (69) provided linker 74 after a 7-step sequence. Linker 74 was then used for the synthesis of hexapeptide 76 in a yield of 70% (Scheme 16).

Scheme 15. Use of photolabile o-nitroveratryl-based linker for the synthesis of oligonucleotides.

In 1997, Holmes published a systematic study of the effect of nitroveratryl substituents on the photolysis efficiency.[53] It was shown that the rate of cleavage was 7 to 20 times higher when the o-nitrobenzyl ring was substituted with electron-donating alkoxy substituents. It was also shown that an alkyl group in the amine α-position increased the rate by a factor of 3. The length of the spacer chain also influenced the release rate with a slight increase for longer chains. It was
concluded that both the alkoxy substituents on the aromatic ring and the alkyl group in the α-position were beneficial for the photochemical reactivity.

Holmes also developed an alcohol-based version of the linker 74,[53] which was synthesized analogously to the corresponding amine linker (Scheme 17).

![Scheme 17. Synthesis of photolabile alcohol-containing α-methylated nitroveratryl linker](image)

Although, this was a useful route for the synthesis of 81, problems were encountered in the nitration of 78, due to contamination with substantial quantities of the ipso substitution of the acetyl group. An improved synthetic pathway to give the ester 80 was developed by Teague.[54] Ipso substitution was substantially reduced, when nitration was performed at low temperature with fuming nitric acid.

The linkers 74 and 81 have been used for numerous applications, and it has been shown that such linkers may be coupled to hydroxyl-based resin without effecting the efficiency of the photolytic cleavage.[55] An o-nitroveratryl linker was used by McKeown et al. to develop methods for easing the analysis of solid-phase synthesis reactions by mass spectrometric techniques.[56] Another example is the use of linker 74 by Madder for the preparation of serine protease mimics.[57] Lin and Blackwell used this linker for the synthesis of a diketopiperazine
library using the Ugi multi-component reaction (Scheme 18).[58] The linker 74 was attached to a solid support and subsequent coupling of Fmoc-Phe(4-NO2)-OH and Fmoc deprotection under standard SPPS conditions led to the construct 82. Ugi reactions, followed by methanolysis and photochemical release afforded diketopiperazines 84 in moderate to good purities.

Scheme 18. o-Nitroveratryl linker used in multicomponent reactions by Lin and Blackwell.
Gennari et al. used the linker 74 for the synthesis of a combinatorial library of vinylogous sulfonamidopeptides.[59] This study confirmed the trends encountered by Holmes,[53] namely that o-nitroveratryl-based linkers are superior to o-nitrobenzyl linkers, due to the beneficial effects of alkoxy-substituents and the presence of a α-methyl group.

Another example is the synthesis of 3,4-disubstituted β-lactams by Gallop and coworkers (Scheme 19).[60] The construct 75 was condensed with an aldehyde to form imines 85 which then underwent a [2+2] cycloaddition with a ketene to provide 86. Finally, photolysis led to β-lactams 87 in good yields (Scheme 19).[60].

![Chemical structures](image)

Scheme 19. α-Methylated nitroveratryl linker used in the synthesis of β-lactams.

Minkwitz and Meldal used the aldehyde linker 88, related to 74, for the synthesis of a small library of arylpiperazine melanocortin subtype-4 agonists.[61] The linker 88 was synthesized and
attached to the solid support (Scheme 20). Construct 89 was then subjected to reductive amination and subsequent acylation of the formed amine afforded 91. Finally, two alkylation steps and photolytic cleavage provided arylpiperazines 94.

Scheme 20. Photolabile linker used for the synthesis of melanocortin subtype-4 agonists.

Qvortrup and Nielsen introduced an azido-functionalized linker based on the o-nitroveratryl group for the synthesis of 1,2,3-trizoles,[62] cf. Scheme 21.
Scheme 21. Photolabile nitroveratryl-based azido-linker used for the synthesis of 1,2,3-triazoles.

The alcohol 95 was synthesized through procedures adapted from Holmes and co-workers (Scheme 17). The alcohol was then subjected to SOCl₂ and after substitution with NaN₃ and ester hydrolysis with LiOH, the azido linker 97 was obtained. Attachment to the solid support and subsequent copper(I)-catalyzed azide–alkyne cycloaddition led to the construct 98, which after photolytic cleavage provided 1,2,3-triazoles 99 in good yields. Nielsen and coworkers later introduced a hydrazine linker.[63] Starting from chloride 96, the linker 100 was synthesized in two steps, and following attachment to the solid support, SPPS and photolytic cleavage provided peptide hydrazides 103 in yields of 21–83% (Scheme 22).
Scheme 22. Photolabile nitroveratryl-based linker used for the synthesis of peptide hydrazides.

The construct 101 could also be utilized for the solid-phase synthesis of dihydropyrano[2,3-c]pyrazoles.[63]

Recently, Qvortrup et al. introduced a photolabile nitroveratryl-based linker 104 for the synthesis and release of hydroxamic acids.[64, 65] Furthermore, they showed, that this linker unit may, uniquely, function as a ‘bidetachable’ system.[64] By simply varying the reaction solvent, the photolysis could be controlled to provide either C-O or C-N bond cleavage, which allowed for controlled release of the hydroxamates 106 or carboxamides 105, respectively (Scheme 23).

Scheme 23. Bi-detachable linker: Solvent-controlled chemoselectivity of phololysis to provide the hydroxamate or carboxamide, respectively.
Another recent example is the linker developed by Lee and co-workers.[66] This linker is tethered to the solid support through the α-position. Starting from veratraldehyde (107) the linker 112 was synthesized in six steps (Scheme 24).

![Scheme 24. Synthesis of Leu-enkephalin amide using photolabile linker 112.](image)

After coupling to the solid support, standard SPPS and photolytic cleavage provided Leu-enkephaline amide (113) in a yield of 50%.

**Phenacyl linkers**

Another useful photo-cleavable linker is based on the phenacyl [PhC(O)CH$_2$-] group. The aryl carbonyl undergoes n→π* or π→π* excitation upon UV radiation (300-350 nm), followed by hydrogen atom abstraction from solvent and bond scission to cleave the linker (Figure 3).[67]
Figure 3. Photochemistry of aromatic ketones that release a leaving group (X).

The phenacyl group was explored in pioneering studies on photolabile linkers by Wang.[68] The resin 1 was subjected to Friedel-Crafts acylation with 2-bromopropionyl chloride, and the first amino acid was O-alkylated to provide construct 115, cf. Scheme 25. Subsequent standard SPPS and final photolysis released the peptide 117 in 70% yield.


A phenacyl linker was also used by Tam to produce resins cleavable under both photolytic, basic or nucleophilic conditions.[12, 13]

Tjoeng and Heavner developed a non-integral phenacyl linker system,[69] which was synthesized in solution and coupled to the resin, to avoid the direct derivatization of the resin as described by Wang. Initial esterification of phenylacetic acid (118) and subsequent Friedel-
Crafts acylation led to the methyl ester 119, which upon simple ester hydrolysis led to the phenacyl linker 120 (Scheme 26). With 120 in hand, attachment to the resin and coupling of the first amino acid, followed by standard SPPS led to the peptide 124 in 78% yield after photolytic release.[68]

Scheme 26. Synthesis of phenacyl photolabile linker 120 and its use for the synthesis of thymopoietin II fragments.

**p-Alkoxyphenacyl linkers**

Introduced by Bellof and Mutter,[70] the p-alkoxyphenacyl linker is structurally similar to the phenacyl linker, but the presence of an electron donating alkoxy group in the para-position induces a bathochromic shift, making the photolysis at 350 nm much more efficient, thus
shortening the irradiation times. The synthesis started from methyl phenoxyacetate (125), which after Friedel-Crafts acylation and ester hydrolysis provided the bromide 126 (Scheme 27). Coupling to the resin followed by attachment of the first amino acid, either in the presence of KF or as its cesium salt, led to 128. Conventional SPPS, photolysis and deprotection led to the peptide 129 in 71% yield.

Scheme 27. Synthesis of a \( p \)-alkoxyphenacyl photolabile linker for peptide synthesis.

Gauthier and co-workers used the same linker for the synthesis of octapeptides in good yield (>85%), as illustrated by the efficient synthesis of mammalian glucagon.[71] Belshaw and co-workers developed a safety-catch photolabile linker based on the \( p \)-alkoxyphenacyl moiety.[72] The photolability of the linker was masked to enable the use of photolabile protecting groups in light-directed synthesis of oligonucleotides. The linker synthesis started from acetophenone 130, (Scheme 28). A Mitsunobu reaction led to 131, which after acetal formation and methyl oxidation provided the alcohol 132. Protection with the photolabile NPPOC group followed by alcohol deprotection gave 133, which was then prepared for attachment to the solid support by treatment with 2-cyanoethyl diisopropylchlorophosphoramidite resulting in the linker 134. Linker
was attached to the solid support and iterative cycles of photolytic deprotection and coupling of nucleotides bearing a photocleavable protecting group furnished construct 136. Mild acidic hydrolysis then activated the linker for photolytic cleavage and final photolysis provided the DNA chain 138.

Sucholeiki introduced a traceless thioether \( p \)-methoxyphenacyl linker for use in Stille cross-couplings.[73, 74] Methylation of 139, Friedel-Crafts acylation and halogen exchange led to the chloride 142 (Scheme 29).

![Chemical reaction diagram]

Scheme 29. Synthesis and use of a traceless thioether \( p \)-methoxyphenacyl linker in Stille cross-coupling reactions.

Substitution and disulfide formation then furnished the linker 143, which after attachment to the solid support and disulfide cleavage was reacted with 4-iodobenzyl bromide to provide construct 145. Pd-catalyzed Stille cross-coupling and subsequent photolysis led to the biaryl 146 in a yield of 27%.

**Benzoin linkers**
The photolysis of 3',5'-diarylbenzoin can be rationalized as a diradical process starting from an $n \rightarrow \pi^*$ transition. A charge transfer interaction of the electron-rich dialkoxyphenyl moiety with the electron deficient oxygen of an $n \rightarrow \pi^*$ singlet excited ketone form an intramolecular exciplex, which after rearrangement, leads to release of the product and formation of the benzofuran (Figure 4).

![Figure 4. Photoinduced cleavage of a Benzoin linker.](image)

Rock and Chan developed a benzoin safety-catch linker for the purpose of binding two molecules together in solution.[75] This was later exploited for solid-phase synthesis by Balasubramanian and co-workers.[76, 77] The synthesis started from 3-hydroxybenzaldehyde (147) which was coupled to the resin and reacted with 2-phenyl-1,3-dithiane to form a dithioacetal, which effectively functioned as a safety-catch moiety that can be triggered upon hydrolysis (Scheme 30). Fmoc-$\beta$-Ala-OH was then coupled to the resin to give 150, which upon hydrolysis of the dithioacetal and photolysis released the amino acid 152 in excellent yields. Good to excellent yields (75–97%) were obtained using this strategy with other carboxylic acids.
Later Balasubramanian and co-workers developed a second-generation, non-integral benzoin linker.\cite{78} The linker was prepared with a carboxylic acid as anchoring point to exploit the wide range of amine functionalized resins available, notably with a long tether, as this had previously been shown to be advantageous for o-nitroveratryl linkers. The synthesis started from aldehyde 153 and a six-step sequence led to the linker 158, cf. Scheme 31.

Scheme 30. Use of photolabile benzoin safety-catch linker in solid-phase synthesis.
Scheme 31. Synthesis of a photolabile benzoin linker with a carboxylic acid anchor.

To address the often challenging dithioacetal removal, Copley and coworkers developed a new benzoin linker in which the carbonyl group was protected as a dimethyl ketal.[79] The dimethyl ketal moiety is stable towards common solid-phase esterification and amide bond formation reaction conditions, yet easily removable with 3% TFA. The synthesis of the linker started from 3-formylbenzoic acid (159), followed by a four-step sequence to provide dithioacetal 163 (Scheme 32). Finally, the dithioacetal functionality was replaced with a dimethyl ketal to provide linker 164. Attachment to the solid support, subsequent SPPS and photolysis released the tetradecapeptide 165.
Scheme 32. Synthesis and use of a photolabile dimethyl ketal benzoin linker for peptide synthesis.

**Pivaloyl linkers**

Pivaloyl derivatives fragment by a Norrish type I mechanism, releasing carboxylic acids or alcohols (Figure 5). The side-products, carbon monoxide and isobutene, are all volatile, making purification simple. However, the process requires the use of a relatively short wavelength source (280–340 nm), which may cause side reactions when applied to more fragile biomolecules.
Peukert and Giese developed a photolabile linker based on the pivaloyl group.[80] The synthesis started from 1,3-dihydroxyacetone dimer (166) and a six-step sequence gave the linker 171 in an overall yield of 40% (Scheme 32). The linker was then coupled to the solid support and following attachment of the first amino acid, standard SPPS provided construct 174, which upon photolysis released the peptide 175 in a yield of 86%.

**Figure 5.** Photolysis of a pivaloyl-based linker.
Scheme 33. Synthesis and use of a pivaloyl-based photolabile linker.

The linker 171 also proved useful for the generation and release of compounds relying on Pd-catalyzed Stille and Suzuki cross-coupling, and epoxidation reactions.[80] Later, a modified linker enabled the release of alcohols upon photolysis.[81]

Bochet and coworkers exploited the property of the pivaloyl linker, used in the synthesis of Leu-Enkephalin, as being stable at longer wavelengths, thus enabling the use of photolabile protection groups with different chromatic lability.[82] The synthesis started with the coupling
of NVOC-Leu-OH to the linker resin 176 under standard conditions (Scheme 34). Four cycles of photolytic deprotection (360 nm) and amino acid coupling reactions furnished construct 178. Finally deprotection and photolytic cleavage from the resin (305 nm) released Leu-enkephalin 179 in a yield of 55%.

Scheme 34. Synthesis of Leu-Enkephalin utilizing both a photolabile linker and photolabile protecting groups.

Other photolabile linkers

Routledge and co-workers introduced a traceless photolabile linker based on thiohydroxamic acid.[83] Starting with attachment of trityl-protected 4-hydroxymercaptophenol 180 to the solid support, the linker construct 184 was prepared in four steps (Scheme 35).
Scheme 35. Synthesis and use of a traceless photolabile linker.

Then N-methylindole-3-acetic acid was attached, and photolysis yielded 1,3-dimethylindole 186 in a yield of 55%. This method, however, suffers from the need of a hydrogen-donor, such as a toxic tin hydride species to facilitate release from the solid support, whereas conventional photolysis normally can be achieved under mild and neutral conditions.

Nicolaou et al. introduced a photolabile linker exploiting the photochemical properties of the 5-bromo-7-nitroindoline group.[84] Photolytic release in the presence of amines provided amides. Intramolecular trapping of an attached amine was also explored in the synthesis of heterocycles. The synthesis started from the indole 187 (Scheme 36).
Scheme 36. A photolabile linker for the synthesis of heterocycles.
Reduction, acylation and methylation led to the methyl ester 189. Nitration followed by ester hydrolysis and amide formation provided the amide 192, which after photolytic removal of the acetyl group and ester hydrolysis furnished the linker 194. Attachment to the solid support and further synthetic elaboration led to the construct 197, which upon photolytic cyclorelease provided the heterocycle 198 in a yield of 50%.

A triazene-based photolabile linker was developed by Enders et al.\[85\] 3-Hydroxyaniline (199) was attached to a Merrifield resin and diazotation followed by reaction with a secondary amine, such as piperazine, led to the triazene construct 202 (Scheme 37).

Scheme 37. Synthesis and use of triazene-based photolabile linker developed by Enders.

Acylation and photolytic release provided the tertiary amide 204. Generally, moderate yields and excellent purities were achieved.

Perspectives

As the interest in synthesizing complex molecules, which may be either base- or acid-sensitive, solid support continues to grow, effective protocols for cleavage under mild, neutral conditions remain extremely appealing for SPOS. Fortunately, photolabile linkers provide unique
opportunities for the release of target molecules under mild, neutral conditions by using UV light. Photochemical cleavage generally proceeds with high functional group selectivity, low amounts of side reactions and involve no post-cleavage purification to separate products from reagents. Furthermore, light-cleavable linkers are stable to most standard chemical conditions used in synthetic transformations and consequently, there is no prematurely cleavage of the library products from the resin beads.

Also, with the development of high-throughput screening techniques, including resin-based bioactivity tests and synthesis of arrays of small molecules on microchips, the importance of photolabile linkers has grown beyond its original purpose. Cleavage can often be performed in aqueous solution, and the liberated products can then be directly employed in biological assays.

The linker of interest may very much depend on the specific application, e.g. some (bio)molecules are not compatible with certain wavelengths and/or specific byproducts. The table below summarizes current photo-cleavable linkers, and the corresponding cleavage conditions. This should prove important for choosing the optimal strategy for a specific application.
<table>
<thead>
<tr>
<th>Linker</th>
<th>Cleavage Conditions</th>
<th>Product</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Nitrobenzyl linkers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Linker Diagram" /></td>
<td>hv, λ = 350 nm</td>
<td>HO-Gly-Tyr-Ser-Boc OBN</td>
<td>[10]</td>
</tr>
<tr>
<td><img src="image" alt="Linker Diagram" /></td>
<td>hv, λ = 350 nm</td>
<td>X = O: Peptides</td>
<td>[11-13, 24, 35]</td>
</tr>
<tr>
<td><img src="image" alt="Linker Diagram" /></td>
<td></td>
<td>X = O: Nucleotides</td>
<td>[20-22]</td>
</tr>
<tr>
<td><img src="image" alt="Linker Diagram" /></td>
<td></td>
<td>X = N: Amido peptides</td>
<td>[32-34, 36]</td>
</tr>
<tr>
<td><img src="image" alt="Linker Diagram" /></td>
<td></td>
<td>X = O: Small molecules</td>
<td>[19, 37]</td>
</tr>
<tr>
<td><img src="image" alt="Linker Diagram" /></td>
<td></td>
<td>X = O: Saccharides</td>
<td>[25-26]</td>
</tr>
<tr>
<td>α-nitrobenzyl linkers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Linker Diagram" /></td>
<td>hv, λ = 350 nm</td>
<td>Amido peptides</td>
<td>[38-40]</td>
</tr>
<tr>
<td><img src="image" alt="Linker Diagram" /></td>
<td></td>
<td>Glycosaminoglycans</td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Linker Diagram" /></td>
<td></td>
<td>Peptides</td>
<td>[41]</td>
</tr>
<tr>
<td><img src="image" alt="Linker Diagram" /></td>
<td></td>
<td></td>
<td>[42]</td>
</tr>
<tr>
<td>α-Nitroveratryl linkers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Linker Diagram" /></td>
<td>hv, λ = 350 nm</td>
<td>Nucleotides</td>
<td>[49],[51-52]</td>
</tr>
<tr>
<td><img src="image" alt="Linker Diagram" /></td>
<td></td>
<td>Saccharides</td>
<td>[48]</td>
</tr>
<tr>
<td><img src="image" alt="Linker Diagram" /></td>
<td>hv, λ = 365 nm</td>
<td>Amido peptides</td>
<td>[50]</td>
</tr>
<tr>
<td><img src="image" alt="Linker Diagram" /></td>
<td></td>
<td>Sulfonamidopeptides</td>
<td>[59]</td>
</tr>
<tr>
<td><img src="image" alt="Linker Diagram" /></td>
<td>hv, λ = 365 nm</td>
<td></td>
<td>[60]</td>
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<td><img src="image" alt="Linker Diagram" /></td>
<td></td>
<td></td>
<td>[62]</td>
</tr>
<tr>
<td><img src="image" alt="Linker Diagram" /></td>
<td>hv, λ = 365 nm</td>
<td></td>
<td>[63]</td>
</tr>
<tr>
<td><img src="image" alt="Linker Diagram" /></td>
<td>hv, λ = 365 nm</td>
<td></td>
<td>[64-65]</td>
</tr>
<tr>
<td><img src="image" alt="Linker Diagram" /></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Linker</td>
<td>Cleavage Conditions</td>
<td>Product</td>
<td>References</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------------</td>
<td>--------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Phenacyl linkers</td>
<td></td>
<td></td>
<td>[67-69]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[70-71]</td>
</tr>
<tr>
<td>Benzoin linkers</td>
<td></td>
<td>Unnatural amino acids</td>
<td>[77]</td>
</tr>
<tr>
<td>Pivaloyl linkers</td>
<td></td>
<td>Small molecules</td>
<td>[80,82]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[81]</td>
</tr>
<tr>
<td>Other photolabile linkers</td>
<td>hv, λ = 290 nm, R1R2NH</td>
<td></td>
<td>[84]</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>[83]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[85]</td>
</tr>
</tbody>
</table>
Table 1. Summary of current photocleavable linker strategies for solid-phase syntheses.

Conclusions

More than 40 years have passed since the first publication of a photolabile linker. A wide range of photolabile linkers has since been developed to provide an extensive toolkit for the synthesis of peptides, oligosaccharides, oligonucleotides and small molecules. Photolabile linkers provide a mode of cleavage, which is orthogonal to reaction conditions normally applied with traditional linkers. Furthermore, these linkers are normally cleaved under very mild conditions, which make them attractive for use to release material directly in buffers used for biological studies,[86] and for synthesis circumventing excessive amounts of acidic cleavage reagents. It is expected that the field will expand alongside emerging lighting technologies, such as LED-based systems, which are now being recognized as unique enablers of photochemical organic reactions.

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ABBREVIATIONS

Acm, acetamido; ANP, 3-amino-3-(2-nitrophenyl)propionyl; BME, β-mercaptoethanol; Bn, benzyl; Boc, tert-butyloxycarbonyl; BOP, benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium
hexafluorophosphate; BrZ, 2-bromobenzyloxy carbonyl; Bzl, benzyl; ClZ, 2-chlorobenzyloxy carbonyl; DCC, N,N'-dicyclohexyl carbodiimide; DCE, dichloroethane; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; DIC, N,N'-diisopropyl carbodiimide; DIEA, N,N-diisopropylethylamine; DKP, diketopiperazine; DMAP, 4-dimethylaminopyridine; DMB, 3',5'-dimethoxybenzoin; DMF, dimethylformamide; DMP, 2,5-dimethylphenacyl; DMTr, 4,4'-dimethoxytrityl; Fmoc, (9-fluorenylmethyl) carbamate; HATU, Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium; HOBt, 1-hydroxybenzotriazole; LCAA-CPG, long chain aminoalkyl controlled pore glass; LED, light-emitting diode; LG, leaving group; MBHA, 4-Methylbenzhydrylamine; NBB, (3-nitrobenzamido)benzyl; NBH, 2-nitrobenzhydrol; NBOC, 2-nitrobenzyloxy carbonyl; NBS, N-bromosuccinimide; NMM, N-methylmorpholine; NMP, N-methylpyrrolidone; NVOC, 6-nitroveratryloxy carbonyl; PEG, polyethylene glycol; PPOA, [4-(2-bromopropionyl)phenoxy]acetic acid; PS, polystyrene; SPPS, solid-phase peptide synthesis; SPOS, solid-phase organic synthesis; TBDPS, tetrabutyldiphenylsilyl; TBTU, O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid; TFP, tri-2-Furylphosphine; THF, tetrahydrofuran; Thy, thymine; TCA, trichloroacetic acid; TLC, thin layer chromatography; Tos, p-toluenesulfonyl; UNP-Gal, uridine diphosphate galactose; Z, carbobenzyloxy (benzyloxy carbonyl).

REFERENCES


[47]: Ryba, T. D.; Harran, P. G. Two Useful Photolabile Surfaces for Solid-Phase Synthesis. 

[48]: Zehavi, U.; Patchornik, A. Oligosaccharide synthesis on a light-sensitive solid support. I. 

[49]: Yoo, D. J.; Greenberg, M. M. Synthesis of Oligonucleotides Containing 3'-Alkyl 
1995*, *60*, 3358–3364.

[50]: Holmes, C. P.; Jones, D. G. Reagents for Combinatorial Organic Synthesis: Development 
2318–2319.

[51]: Venkatesan, H.; Greenberg, M. M. Improved Utility of Photolabile Solid Phase Synthesis 

[52]: Poijaervi, P.; Heinonen, P.; Virta, P.; Loennberg, H. 2,2-Bis(ethoxycarbonyl)- and 2-
(Alkylaminocarbonyl)-2-cyano-Substituted 3-(Pivaloyloxy)propyl Groups as Biodegradable 

[53]: Holmes, C. P. Model Studies for New o-Nitrobenzyl Photolabile Linkers: Substituent 


**Graphical Abstract:**

*Photolabile Linkers for Solid-Phase Synthesis*

[Diagram of photolabile linker synthesis]

 ACS Paragon Plus Environment
NO2
LG
ν
NO
O
H-LG

R
R
+
N
R
O
O
N
LG
OH
LG

aci-nitro compound

hv

hydrogen abstraction

R

NO
O
H-LG

R

O

R

OH

H-LG
1. SnCl₄, ClCH₂OMe
2. HNO₃

Boc-Gly-OH, Et₃N

O₂N

Boc-Ser(Bn)-Tyr(Bn)-Gly

hv (350 nm)

MeOH

62%

O₂N

Boc-Ser(Bn)-Tyr(Bn)-Gly-OH

Polystyrene resin

ACS Paragon Plus Environment
1. SPPS
2. hv (350 nm), MeOH

pGlu-His(Bn)-Trp-Ser(Bn)-Tyr(Bn)-Gly-Leu-Arg(Ts)-Pro-Gly-OH

64%

: polystyrene resin
Gly-Gly-Phe-Leu

photolabile

acid labile

thiol labile

: polystyrene resin

12
1. DCC, CH$_2$Cl$_2$
2. HOBr, H$_2$O, H$_2$N-DMF

: LCAA-CPG

13 + 14 → 15
1. deprotection
2. EDCI, PLL
   DMF:H₂O (2:1)

UDP-Gal, D-galactosyltransferase
sodium cacodylate buffer

D-Gal(β1→4)D-Glc(β1→4)D-Glc(β1→4)

D-Gal(β1→4)D-Glc(β1→4)D-Glc(α/β)

0.87% :

polyacrylamide gel resin
1. NBS, benzene
2. HNO₃

1. TFA:CH₂Cl₂ (1:1)
2. Boc-Val-OH, DCC

: aminomethyl polystyrene resin
1. NBS, benzene
2. HNO3

1. NH₃ (liq.)
2. BocN₃

BocHN

1. TFA:CH₂Cl₂ (1:1)
2. Boc-Val-OH, DCC

ν (350 nm)

DMF

MeOH

NH₂

quant.

: aminomethyl polystyrene resin
Figure 1. Benzyl 2-[(1H-tetrazol-5-yl)carbonyl]ethyleneglycol ether (36) was used as a linker residue for the initial addition of resin with the carbohydrate building blocks 37 (left) and 38 (right). The resin was then coupled to generate 41 containing the linker. The target carbohydrate 40 (13%) was isolated after final modifications, deprotection of temporary protection group and photolysis.
1. AcCl, AlCl$_3$, nitrobenzene
2. NaBH$_4$, diglyme

1. HBr (g), CH$_2$Cl$_2$
2. HNO$_3$

Boc-Glu(OBn)-OH, DIPEA
EtOAc

Boc-Asp(OBn)-Val-Tyr(Bn)-Val-Glu(OBn)-OH

hv (320 nm)
EtOH:CH$_2$Cl$_2$ (1:1)

Boc-Asp(OBn)-Val-Tyr(Bn)-Val-Glu(OBn)-OH

46 40%

: polystyrene resin
1. HBr
2. NH₃

peptide OH

1. SPPS
2. hν, MeOH:DMF(1:1)

peptide NH₂: polystyrene resin

peptide—NH₂
61–78%

51

peptide—OH
56–60%

49

1. SPPS
2. hν, EtOH

50

1,2-DCE diglyme

47

48
peptide—NH₂
51
61–78%

1. HBr
2. NH₃
3. SPPS
4. hv, MeOH:DMF(1:1)

peptide—OH
49
56–60%
**Reaction Scheme**

1. **Compound 56**
   - Reaction with **NO₂BF₄** and **CH₃NO₂**

2. **Compound 57**
   - Reaction with **NaN₃**, **H₂SO₄**, and **toluene**

3. **Compound 58**
   - Reaction with **(Boc)₂O**, **DMAP**, and **THF**

4. **Compound 59**
   - Reaction with **m-CPBA**, **NaHPO₄**, and **CH₂Cl₂**

5. **Compound 60**
   - Reaction with **LiOH**, **THF**:**H₂O (2:1)**
   - Where **R = OH**

6. **Compound 61**
   - Reaction with **LiOH**, **THF**:**H₂O (1:1)**
   - Where **R = NHBoc**
1. \( \text{Br(CH}_2\text{)}_3\text{OH, K}_2\text{CO}_3 \) 
\( \text{MeCN} \)
2. \( \text{HNO}_3, \text{AcOH} \)

\[
62 \xrightarrow{1. \text{Br(CH}_2\text{)}_3\text{OH, K}_2\text{CO}_3 \text{ MeCN}} \xrightarrow{2. \text{HNO}_3, \text{AcOH}} 63
\]

1. \( \text{K}_2\text{CO}_3, \text{MeOH} \)
2. \( \text{TBDMSCl, Im, DMF} \)
3. \( \text{NaBH}_4, \text{EtOH} \)

\[
63 \xrightarrow{1. \text{K}_2\text{CO}_3, \text{MeOH}} \xrightarrow{2. \text{TBDMSCl, Im, DMF}} \xrightarrow{3. \text{NaBH}_4, \text{EtOH}} 64
\]
1. TBAF, AcOH, THF
2. PDC, DMF
3. 2,4,6-trichlorophenol, DCC

1. oligonucleotide synthesis
2. \( h \nu (400 \text{ nm}), \text{MeCN:H}_2\text{O} (9:1) \)
3. detritylation (\( \text{NH}_4\text{OH, aq} \))

\[ \text{5'-TACGCAATCCTAGATCTAAT} \]
\[ \text{P(OCH}_2\text{)}_3\text{COOH} \]

80%
1. Br(CH$_2$)$_3$COOMe, K$_2$CO$_3$, DMF
2. NH$_2$OH·HCl, pyridine:H$_2$O (2:1)

1. H$_2$, Pd/C, AcOH
2. TFAA, pyridine

1. H$_2$N—, HATU, DIPEA, DMF
2. 20% piperidine (DMF)

1. SPPS
2. $h\nu$(365 nm), PBS buffer:DMSO (95:5)

H-Met-Gly-Trp-Met-Asp-Phe-NH$_2$

70%
OH
MeO
O
77
Br(CH₂)₃COOMe, K₂CO₃, DMF

O
MeO
O
78
HNO₃, AcOH

O
MeO
O
79
NO₂
NaOH, MeOH

O
MeO
O
80
NO₂
NaBH₄, THF

O
MeO
O
81
NO₂
NaOH, MeOH
1. DIC, HOSu, DIPEA, NMP, MW
2. 20% piperidine (DMF)

1. DIC, HOSu, DIPEA, NMP, MW
2. 20% piperidine (DMF)

1. 10% AcCl (MeOH)
2. 20% piperidine (DMF)
3. h\nu (366 nm), MeOH

53–91% purity

R:\ O2N
R2: amino acid side chain
R3: Cy, CH3(CH2)5, Ph(CH2)2
R4: modified cellulose
R<sup>1</sup>CHO,
(MeO)<sub>3</sub>CH:CH<sub>2</sub>Cl<sub>2</sub> (1:1)

R<sup>1</sup> = COOt-Bu, 2-pyridyl, Ph<sub>2</sub>CCH

R<sup>2</sup>CH<sub>2</sub>COCl, Et<sub>3</sub>N

hv (365 nm)

DMSO

71–90%

TentaGel resin
1. Br(CH₂)₃COOMe, K₂CO₃, DMF
2. HNO₃
3. NaOH, MeOH

1. R¹NH₂
2. NaCNBH₃
DMF:MeOH:AcOH (9:9:2)

1. 1R¹NH₂
2. NaCNBH₃
DMF:MeOH:AcOH (9:9:2)

1. R¹NH₂
2. NaCNBH₃
DMF:MeOH:AcOH (9:9:2)

1. R¹NH₂
2. NaCNBH₃
DMF:MeOH:AcOH (9:9:2)

12–35%

R¹HNCH₂NR²

: PEGA₈₀₀
1. **NaN₃**, DMF
2. LiOH, THF:H₂O (3:7)

\[ \text{MeO} \quad \text{O} \quad \text{MeO} \quad \text{O} \quad \text{96} \text{OEt} \quad \text{OEt} \]

1. TBTU, NEM, DMF
2. CuI, 2,6-lutidine, sodium ascorbate, NMP:H₂O (1:1)

\[ \text{MeO} \quad \text{O} \quad \text{MeO} \quad \text{O} \quad \text{98} \text{NH} \]

\[ \text{R} \quad \text{h} \quad \text{(365 nm)} \quad \text{MeOH:H₂O (1:4)} \]

\[ \text{N} \quad \text{N} \quad \text{R} \quad \text{99} \text{HN} \]

\[ \text{R} \quad \text{51–77%} \]

\[ \text{PEG₈₀₀} \]
HiCore resin

107

O

OMe

malonic acid,
NH₄OAc
EtOH

H₂N

OMe

OMe

H₂N

AcCl
MeOH

Cl⁻
H₃N

OMe

OMe

H₃N

AcCl
MeOH

pyridine

108

109

110

F₃COCHN

OMe

O

1. NaOH

O₂N

111

1. H₂N
BOP, HOBt,
DIPEA, DMF

2. SPPS

3. hν (365 nm), MeOH

H-Tyr-Gly-Gly-Phe-Leu-NH₂

112

FmocHN

OMe

O

113

50%

FmocOSu,
DIPEA, THF

SPPS
\[ \text{hv} \rightarrow \text{H-abstraction} \]

\[
\begin{array}{c}
\text{substrate} \\
\text{product} + \text{HX}
\end{array}
\]
2-bromopropionyl chloride, \( \text{AlCl}_3 \)

\[ \text{CH}_2\text{Cl}_2 \]

\[ \text{DMF} \]

\[ \text{2-bromopropionyl chloride, AlCl}_3 \]

\[ \text{CH}_2\text{Cl}_2 \]

\[ \text{DMF} \]

\[ h_v \ (350 \text{ nm}) \]

\[ \text{DMF} \]

\[ : \text{polystyrene resin} \]
1. MeOH, H$_2$SO$_4$

2. 2-bromopropionyl chloride, AlCl$_3$, Cl$_2$CHCHCl$_2$

HCl:H$_2$O:acetone (15:35:50)

H$_2$N$\rightarrow$ DCC

Boc-Tyr(Bzl)-OH/KF

DMF

SPPS

Z-Arg(Z,Z)-Lys(Z)-Asp(OBzl)-Val-Tyr(Bzl)-OH

DMF

hv (350 nm)

Z-Arg(Z,Z)-Lys(Z)-Asp(OBzl)-Val-Tyr(Bzl)-OH

$78\%$

: aminomethyl resin
1. SPPS
2. $\text{hv} (350 \text{ nm}), \text{DMF}$
3. deprotection

125 \[ \text{2-bromopropionyl chloride, AlCl}_3, \text{CHCl}_2 \text{CH}_2 \text{Cl} \]

2. $\text{HBr:acetone:H}_2\text{O}$ (1:4:2)

126

127

128

H-Tyr-Gly-Gly-Phe-Leu-OH

71%: aminomethyl polystyrene resin
1. KOH, MeOH
2. PhI(OAc)_2, MeOH

1. NPPOCCI, pyridine, CH2Cl2
2. TBAF, THF

cycles of photolytic deprotection and nucleotide coupling

3% Cl3CCOOH acetone:MeOH (95:5)

monohydroxy glass slide
TentaGel

139

O

140

O

2-bromopropionyl
chloride, AlCl₃

OMe

OMe

141

O

OMe

OMe

HCl:H₂O:
acetone

142

OH

1. t-BuSH, NaH, THF

2. 2-nitrophenylsulfenyl
chloride, AcOH:DMF:H₂O

OMe

OMe

143

O

144

O

DIC

hν (350 nm), MeCN

145

27%

1. Me₃SnPh, Pd₂(dba)₃,
LiCl, TFP, NMP

2. hν (350 nm), MeCN

146

: TentaGel
\[
\text{O} \quad \text{OH} \\
\text{Cl} \\
\text{DMF} \\
\text{O} \\
\text{O} \\
\text{S} \text{S} \\
\text{Ph} \\
\text{BuLi} \\
\text{THF} \\
\text{O} \\
\text{O} \\
\text{S} \text{S} \\
\text{Ph} \\
\text{Fmoc-} \text{β-Ala-OH, DIC, DIPEA, HOBt, DMAP} \\
\text{DMF} \\
\text{O} \\
\text{NHFmoc} \\
\text{MeOTf} \\
\text{CH}_2\text{Cl}_2 \\
\text{O} \\
\text{h} \nu (350 \text{ nm}) \\
\text{THF:MeOH (3:1)} \\
\text{Fmoc-} \text{β-Ala-OH} \\
\text{152} \\
\text{98%} \\
\text{Merrifield resin}
\]
1. SEMCl, DIPEA, CH₂Cl₂ → Me₃Si-CH₂-O-CH₂-O-Me₂Cl → Me₃Si-CH₂-O-CH₂-O-Me₂Cl

2. Ph, n-BuLi, THF → 154

154 → OAc → HO

3. AcCl, pyridine → 155

4. Ph, n-BuLi, THF

154 → OAc → HO

5. K₂CO₃, Et₃N, MeOH:H₂O (1:1) → 156

6. HCOOH → 157

7. K₂CO₃, Bu₄NI → MeCN → 155

8. HCOOH → 157
1. H₂N, PyAOP, HOAt, DIPEA, DMF
2. SPPS
3. hv, MeOH:H₂O (3:7)

\[ \text{H-Lys-Tyr-Arg-Arg-Pro-Arg-Arg-Ser-Gln-Arg-Lys-Arg-Gly-OH} \]

\[ \text{BTI: [bis(trifluoroacetoxyl)iodo]benzene} \]

\[ \text{TentaGel resin} \]
1. t-BuLi, CuI
2. AcOH

1. K₂CO₃, MeOH
2. BrCH₂COOH-t-Bu, Ag₂O,
DMF

1. DIC, H₂N-, CH₂Cl₂
2. (HF)₃-νOE₃N-
THF

Fmoc-Leu-anhydride,
DMAP

hν (300–400 nm)

Fmoc-Tyr(t-Bu)-Gly-Gly-Phe-Leu-OH

86%
1. $h_v$ (360 nm), MeOH
2. NVOC-AA-OH, DIC, HOBt, CH$_2$Cl$_2$:DMF (10:3)

H-Tyr-Gly-Gly-Phe-Leu-OH

55%
180
\[
\text{ST}_{\text{OH}} \xrightarrow{\text{Cl}^- \text{NaH} \text{DMF}} \text{ST}_{\text{O}}
\]

181
\[
\text{ST}_{\text{O}} \xrightarrow{\text{TFA:CH}_2\text{Cl}_2\text{Et}_3\text{SiH (9:10:1)}} \text{HS}_{\text{O}}
\]

182
\[
\text{HS}_{\text{O}} \xrightarrow{\text{TCDI CH}_2\text{Cl}_2} \text{O}
\]

183
\[
\text{N-S-S-N} \xrightarrow{\text{CH}_3\text{NHOH-HCl, Et}_3\text{N CH}_2\text{Cl}_2} \text{N-S-S-N}
\]

184
\[
\text{N-S-S-N} \xrightarrow{\text{DIC, HOBT, DMAP CH}_2\text{Cl}_2\text{DMF}} \text{O}
\]

185
\[
\text{N-S-S-N} \xrightarrow{h\nu (350 \text{ nm}) \text{THF:Bu}_3\text{SnH (40:1)}} \text{O}
\]

186
\[
55\%
\]

: chloromethyl-polystyrene resin
1. NaCNBH₃, AcOH
2. Ac₂O, HCl

187

\[
\text{AcOH} \quad 188 \quad \text{Me}, Cs₂CO₃, \text{DMF} \quad 189
\]

HNO₃, ClCH₂CH₂Cl →

\[
\text{AcNO}_2 \quad 190 \quad \text{LiOH, } H₂O: THF (1:1) \quad 191
\]

\[
\text{AcNO}_2 \quad 190 \quad \text{EtO}_2C(CH₂)₃NH₃Cl, EDC, HOAt, \text{DMF} \quad 191
\]

H₂O:THF (1:1)

192

\[
\text{Me} \quad 193
\]

\[
\text{H}_2\text{O}:\text{THF (1:1)} \quad 194
\]

\[
\text{DIC, HOAt, DMF} \quad 195
\]

1. BocNH(CH₂)₂CO₂H, TBAI, CS₂CO₃, DMF
2. TFA:CH₂Cl₂ (1:3)

\[
\text{H}_2\text{N} \quad 196
\]

\[
\text{h} (>290 \text{ nm}) \quad 197
\]

\[
\text{THF} \quad 198
\]

: aminomethyl polystyrene resin

188
189
190
191
192
193
194
195
196
197
198
50%
H₂N-OH  199  Cl⁻, NaH, DMF  H₂N-O-  200  t-BuONO, BF₃·Et₂O, THF  BF₄⁻  201

H₂N-O-  200  piperazine  N₂N₂N  202  CyCOCl  N₂N₂N  203

hν (355 nm)  204

Et₂O:MeOH

: Merrifield resin
<table>
<thead>
<tr>
<th>Linker</th>
<th>Cleavage Conditions</th>
<th>Product</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>o-Nitrobenzyl linkers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="linker1.png" alt="Linker" /></td>
<td>( h, \lambda = 350 \text{ nm} )</td>
<td>OBn</td>
<td>[10]</td>
</tr>
<tr>
<td><img src="linker2.png" alt="Linker" /></td>
<td>( h, \lambda = 350 \text{ nm} )</td>
<td>X = O: Peptides, Nucleotides, Amido peptides, Small molecules, Saccharides</td>
<td>[11-13, 24, 35], [20-22], [32-34, 36], [25-26]</td>
</tr>
<tr>
<td>o-nitrobenzyl linkers</td>
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<tr>
<td><img src="linker3.png" alt="Linker" /></td>
<td>( h, \lambda = 350 \text{ nm} )</td>
<td>Amido peptides, Glycosaminoglycans</td>
<td>[38-40]</td>
</tr>
<tr>
<td><img src="linker4.png" alt="Linker" /></td>
<td>( h, \lambda = 350 \text{ nm} )</td>
<td>Peptides</td>
<td>[41], [42]</td>
</tr>
<tr>
<td>o-Nitroveratryl linkers</td>
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<tr>
<td><img src="linker5.png" alt="Linker" /></td>
<td>( h, \lambda = 350 \text{ nm} )</td>
<td>Nucleotides, Saccharides</td>
<td>[49, 51-52], [46]</td>
</tr>
<tr>
<td><img src="linker6.png" alt="Linker" /></td>
<td>( h, \lambda = 365 \text{ nm} )</td>
<td>Amido peptides, Sulfonamidopeptides</td>
<td>[50], [59]</td>
</tr>
<tr>
<td><img src="linker7.png" alt="Linker" /></td>
<td>( h, \lambda = 365 \text{ nm} )</td>
<td></td>
<td>[60]</td>
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<tr>
<td><img src="linker8.png" alt="Linker" /></td>
<td>( h, \lambda = 365 \text{ nm} )</td>
<td></td>
<td>[62]</td>
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<td><img src="linker9.png" alt="Linker" /></td>
<td>( h, \lambda = 365 \text{ nm} )</td>
<td></td>
<td>[63]</td>
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<tr>
<td><img src="linker10.png" alt="Linker" /></td>
<td>( h, \lambda = 365 \text{ nm} )</td>
<td></td>
<td>[64-65]</td>
</tr>
<tr>
<td>Linker</td>
<td>Cleavage Conditions</td>
<td>Product</td>
<td>References</td>
</tr>
<tr>
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<tr>
<td>Phenacyl linkers</td>
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<td>[67-69]</td>
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<td><img src="image1" alt="Phenacyl linker structure" /></td>
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<td>Peptides</td>
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</tr>
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<td>$h\nu, \lambda = 300-350 \text{ nm}$</td>
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<td>Peptides</td>
<td>[70-71]</td>
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<tr>
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<td>$h\nu, \lambda = 350 \text{ nm}$</td>
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<tr>
<td>Benzoin linkers</td>
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<td>[77]</td>
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<tr>
<td><img src="image2" alt="Benzoin linker structure" /></td>
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<td>Unnatural amino acids</td>
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</tr>
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<td>$h\nu, \lambda = 350 \text{ nm}$</td>
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<tr>
<td>Pivaloyl linkers</td>
<td></td>
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<td>[80,82]</td>
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<tr>
<td></td>
<td></td>
<td>Peptides</td>
<td>[81]</td>
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<tr>
<td><img src="image3" alt="Pivaloyl linker structure" /></td>
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<td>Small molecules</td>
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<td>$h\nu, \lambda = 280-340 \text{ nm}$</td>
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<tr>
<td>Other photolabile linkers</td>
<td></td>
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<td>[84]</td>
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<tr>
<td><img src="image4" alt="Other photolabile linker structure" /></td>
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<td>$R^1R^2\text{NH}$</td>
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<td>$h\nu, \lambda = 290 \text{ nm}$</td>
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<td><img src="image5" alt="Other photolabile linker structure" /></td>
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<td>$R^1N^1\text{O}^1\text{Ph}$</td>
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<td></td>
<td>$h\nu, \lambda = 350 \text{ nm}$</td>
<td>$\text{BnSnH, THF}$</td>
<td>[83]</td>
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<tr>
<td><img src="image6" alt="Other photolabile linker structure" /></td>
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<td>$\text{N}$</td>
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<tr>
<td></td>
<td>$h\nu, \lambda = 355 \text{ nm}$</td>
<td></td>
<td>[85]</td>
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</tbody>
</table>

ACS Paragon Plus Environment
Photolabile Linkers for Solid-Phase Synthesis

= Solid support

hv