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A Photolabile Linker for the Solid-Phase Synthesis of Peptide Hydrazides and Heterocycles

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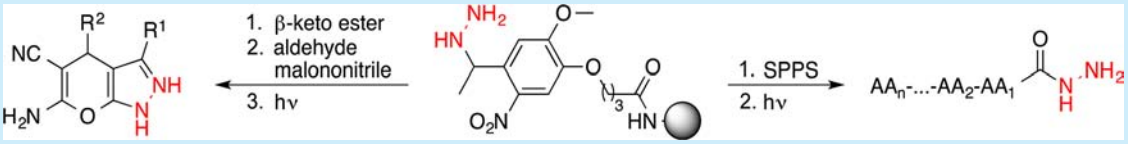
Supporting Information



ABSTRACT: A photolabile hydrazine linker for the solid-phase synthesis of peptide hydrazides and hydrazine-derived heterocycles is presented. The developed protocols enable the efficient synthesis of structurally diverse peptide hydrazides derived from the standard amino acids, including those with side-chain protected residues at the C-terminal of the resulting peptide hydrazide, and are useful for the synthesis of dihydropyrano[2,3-*c*]pyrazoles. The linker is compatible with most commonly used coupling reagents and protecting groups for solid-phase peptide synthesis.

Organic hydrazides are versatile building blocks for the synthesis of pharmacologically relevant heterocycles and advanced materials.[1,2] Peptide hydrazides have been widely utilized in bioconjugation reactions to form glycoconjugates[3] and more recently emerged as powerful precursors for peptide ligation.[4] However, their applicability may be limited in two respects: (1) peptide hydrazides remain challenging to synthesize and require postsynthesis purification prior to ligation and (2) current peptide hydrazide synthesis strategies are not compatible with all amino acid residues or their side-chain protected derivatives. Therefore, more efficient and mild methods for peptide hydrazide synthesis are needed.

Given the importance of hydrazide derivatives in drug and probe discovery efforts, the solid-phase synthesis of protected hydrazides have been subject to many studies. Peptide hydrazides may be directly accessed from solid-supported ester-linked peptides by hydrazinolysis,[5] but this strategy often requires an excess of hydrazine, which is incompatible with a range of protecting groups.[6] The method also complicates postcleavage purification and gives low yields of peptides containing side-chain protected cysteine, aspartic or glutamic acid residues.[7] Wang et al. reported the synthesis of peptide hydrazides on alkoxycarbonyl hydrazide resins.[8] Recent strategies have relied on 2-Cl-trityl-derived hydrazine resins,[9] where postsynthetic release of compounds can be achieved with more dilute TFA solutions. These linkers nevertheless face several limitations, as C-terminal glutamine, asparagine, or aspartic acid residues are generally not tolerated[4a,10] and some reported peptide hydrazides remain challenging to synthesize using these methods.[4f] Acidic reactions can generally not be employed to elaborate the solid-supported hydrazides, and many protected derivatives are not compatible with acids.

Photolysis offers an exceedingly mild method for releasing compounds from solid supports which is fully orthogonal to chemical methods, and attractive for direct applications in biochemical reactions, where contamination with cleavage reagents is undesired. We now wish to report a photolabile linker based on the *o*-nitro-veratryl group,[11] which is capable of releasing peptide hydrazides upon UV irradiation. The strategy is fully orthogonal to the most commonly used protecting groups and chemical methods in SPPS and shows excellent compatibility with peptide composition; notably all 20 natural occurring α-amino acid residues (including side-chain protected analogs) are accepted in the C-terminal of the peptide hydrazides. Furthermore, this linker unit can be applied to synthesize combinatorial libraries of biological interesting heterocyclic compounds, such as pyranopyrazoles.

The synthesis of the linker commenced with the alkylation of acetovanilone with ethyl-4-bromobutyrate followed by nitration, reduction, and chlorination to yield key intermediate benzylic chloride **2** in 70% overall yield (Scheme 1).[12] Substitution of chloride with *tert*-butyl carbazate gave **3**, and finally, hydrolysis of the ester moiety afforded the linker **1**. It can be made in only six steps with an overall yield of 59% (+10 g scale).

Whereas the linker **1** was readily coupled[13] to an amino-functionalized solid support (ChemMatrix), synthetic difficulties were encountered in the removal of the Boc-group. Exposing the immobilized linker to standard TFA/CH_2Cl_2 (1:1) deprotection conditions resulted in formation of the corresponding trifluoroacetylated derivative. By screening a variety of acidic reaction conditions, we found that TMSOTf/2,6-lutidine[14] mediated a clean Boc-deprotection to give the resin-bound hydrazine-derivative **4**. Peptides were then synthesized via an Fmoc-strategy using TBTU/NEM-mediated

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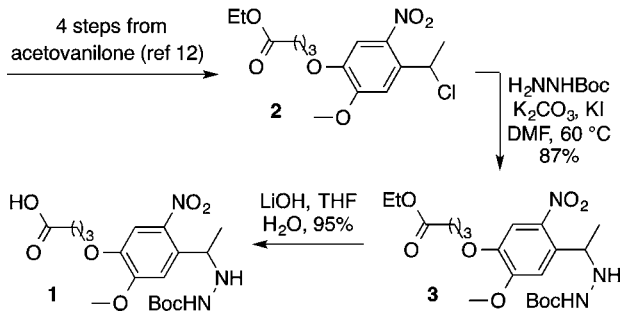
Scheme 1. Synthesis of Boc-Protected Hydrazine Photolabile Linker 1

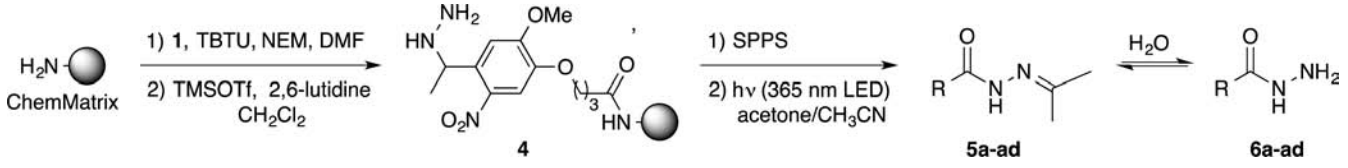


coupling reactions, and photolytic cleavage was routinely carried out by irradiating for 60 min at rt with 365 nm light using an LED UV-lamp. To avoid any side reactions associated with the released hydrazide functionality, including reactions with the resin-bound nitroso and ketone groups formed in the event of photolysis, the release was performed in acetone/CH_3CN (3:2), thereby conveniently releasing the peptide hydrazide protected as its acetone hydrazone. No separate deprotection step was required to free the hydrazide, as the acetone moiety rapidly dissociates under the aqueous conditions commonly used in ligation and bioconjugation reactions. Evaporation of the aqueous solution of acetone hydrazone cleanly provided the free hydrazide without the need for postcleavage purification. Rewardingly, the 20 standard amino acid residues (including side-chain protected analogs) were accepted in the C-terminal position of the peptide hydrazide (Table 1). Peptide hydrazides containing C-teminal Glu(tBu) and Asp(*t*Bu) residues should be released just prior to use, or stored as an acetone solution, as storage of the free peptide hydrazides over a longer time resulted in conversion into the corresponding 1,2-diazepane-3,7-diones and tetrahydropyridazine-3,6-diones, respectively.

Given the importance of peptide hydrazides in protein chemical synthesis, we envisioned the application of linker **1** in an efficient one-pot strategy, comprising solid-phase synthesis, mild photolytic release, and the direct ligation of the crude peptide hydrazides to cysteine-containing peptides. Peptide hydrazide **6i** (Table 1, entry 9) was easily synthesized from **4** and cleanly released as the acetone hydrazone by light. After removal of the excess acetone by a stream of nitrogen, an aqueous phosphate (0.2 M) buffer contaning 6.0 M guanidinum chloride followed by H-Cys-OH was added (final peptide concentration of 1.5 and 2.0 mM, respectively). At low pH (3.0) and temperature (−10 °C), an aqueous $NaNO_2$ solution was added to the ligation mixture. After 20 min, 4-mercaptophenylacetic acid was added, the pH value was adjusted to 7.0, and the reaction was left at room temperature for 12 h. Analysis of the reaction by HPLC showed a clean ligation reaction in 85% yield. Utility of the linker is illustrated by the synthesis of the decapeptide H-Leu-Tyr-Arg-Ala-Tyr-Cys-Lys-Tyr-Met-His-OH (**8**) through ligation of peptide hydrazide **6i** with peptide fragment **7** using our one-pot protocol (Scheme 2). We found that the ligation proceeded as cleanly as reported by Liu and co-workers[4a] to provide the desired product **8** in a high yield (93%).

After the successful preparation of peptide **8**, we focused our attention on the application of the same strategy to ligate the more challenging peptides H-Leu-Tyr-Arg-Ala-Asn-$NHNH_2$

Table 1. Synthesis and Photolytic Release of Peptide Hydrazides (6a−6ad)



entry	C-terminal AA	peptide hydrazide	purity[a]	yield	entry	C-terminal AA	peptide hydrazide	purity[a]	yield
1	Gly	naphtoyl-Phe-Gly-$NHNH_2$ (**6a**)	>95%	34%	16		Fmoc-Arg(Pbf)-Ala-Asn(Trt)-$NHNH_2$ (**6p**)	>95%	36%
2	Ala	naphtoyl-Ala-$NHNH_2$ (**6b**)	82%	36%	17		H-Leu-Tyr(O*t*Bu)-Arg(Pbf)-Ala-Asn(Trt)-$NHNH_2$ (**6q**)	>95%	42%
3	Val	Boc-Phe-Val-$NHNH_2$ (**6c**)	>95%	44%	18	Gln	Fmoc-Ala-Gln(Trt)-$NHNH_2$ (**6r**)	90%	31%
4	Leu	Boc-Pro-Phe-Leu-$NHNH_2$ (**6d**)	89%	48%	19		H-Leu-Tyr-Arg-Ala-Gln-$NHNH_2$ (**6s**)	>95%	31%
5	Ile	Boc-Phe-Ala-Ile-$NHNH_2$ (**6e**)	87%	59%	20	Cys	Boc-Phe-Leu-Cys(S*t*Bu)-$NHNH_2$ (**6t**)	>95%	42%
6	Met	Boc-Leu-Phe-Met-$NHNH_2$ (**6f**)	87%	44%	21	Arg	Boc-Ala-Tyr(*t*Bu)-Arg(Pbf)-$NHNH_2$ (**6u**)	>95%	43%
7	Phe	naphtoyl-Leu-Phe-$NHNH_2$ (**6g**)	>95%	47%	22	His	Boc-Val-Phe-His(Boc)-$NHNH_2$ (**6v**)	90%	52%
8	Pro	naphtoyl-Phe-Pro-$NHNH_2$ (**6h**)	>95%	83%	23		naphtoyl-His-$NHNH_2$ (**6w**)	>95%	55%
9	Tyr	H-Leu-Tyr-Arg-Ala-Tyr-$NHNH_2$ (**6i**)	>95%	58%	24	Lys	Boc-Ala-Phe-Lys(Boc)-$NHNH_2$ (**6x**)	94%	55%
10		Boc-Leu-Tyr(*t*Bu)-Arg(Pbf)-Ala-Tyr(*t*Bu)-$NHNH_2$ (**6j**)	>95%	20%	25		Fmoc-Tyr(O*t*Bu)-Arg(Pbf)-Ala-Asp(O*t*Bu)-$NHNH_2$ (**6y**)	>95%	51%
11	Trp	Boc-Phe-Ala-Trp-$NHNH_2$ (**6k**)	>95%	44%	26	Asp	H-Leu-Tyr-Arg-Ala-Asp-$NHNH_2$ (**6z**)	85%	56%
12	Ser	Boc-Phe-Ser(Bzl)-$NHNH_2$ (**6l**)	>95%	43%	27		Boc-Phe-Asp(*t*Bu)-$NHNH_2$ (**6aa**)	>95%	67%
13	Thr	Boc-Phe-Leu-Thr(*t*Bu)-$NHNH_2$ (**6m**)	87%	42%	28		H-Leu-Tyr(O*t*Bu)-Arg(Pbf)-Ala-Asp(O*t*Bu)-$NHNH_2$ (**6ab**)	>95%	59%
14	Asn	Boc-Ala-Tyr(*t*Bu)-Asn(Trt)-$NHNH_2$ (**6n**)	92%	44%	29	Glu	H-Leu-Tyr(O*t*Bu)-Arg(Pbf)-Ala-Glu(O*t*Bu)-$NHNH_2$ (**6ac**)	85%	60%
15		H-Leu-Tyr-Arg-Ala-Asn-$NHNH_2$ (**6o**)	80%	30%	30		Fmoc-Ala-Glu(O*t*Bu)-$NHNH_2$ (**6ad**)	90%	61%

[a]The purity of the combined peptide hydrazide and corresponding acetone hydrazone was determined by RP-HPLC and UPLC-MS.

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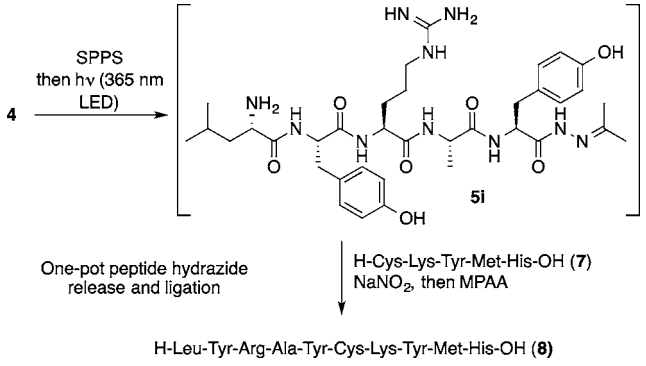
Scheme 2. Application of Linker Construct 4 for the Synthesis of a Decapeptide

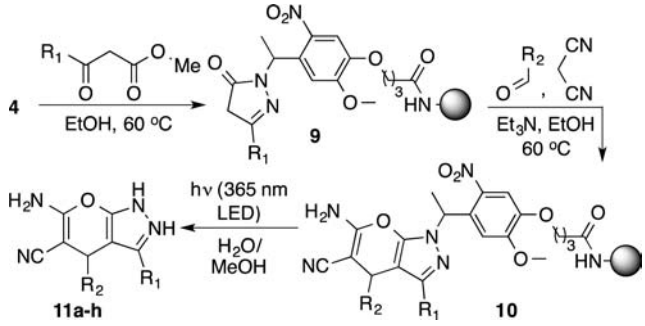


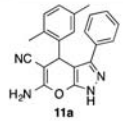
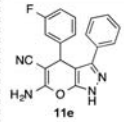
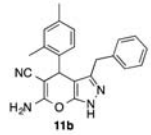
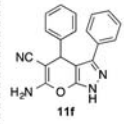
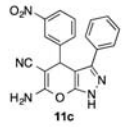
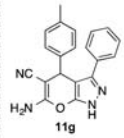
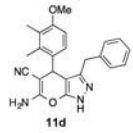
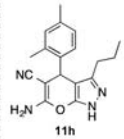
(**6o**), H-Leu-Tyr-Arg-Ala-Gln $NHNH_2$ (**6s**), and H-Leu-Tyr-Arg-Ala-Asp-$NHNH_2$ (**6z**) with H-Cys-OH. However, although we showed the successful release of these peptides (protected as there acetone hydrazones) in excellent yield (Table 1, entries 15, 19, 26), the acidic conditions used in the ligation resulted in reaction mixtures containing the desired ligation product and the side product resulting from an intramolecular cyclization reaction between the hydrazide and the C-terminal side-chain amide or acid group. While we were able to obtain a ligation yield of 55% with H-Leu-Tyr-Arg-Ala-Gln-$NHNH_2$ (**6s**), the Asp C-terminal peptide hydrazide **6z** resulted in a much lower yield (<20%), and we were not able to detect any ligation product in the case of **6o** (Asn C-terminal).

To further demonstrate the synthetic potential of the linker **1**, the synthesis of more elaborate heterocycles was investigated. It was decided to target dihydropyrano[2,3-*c*]pyrazoles, which constitute an important class of compounds with nummerous biological properties, such as anticancer,[15] antimicrobial,[16] and antiinflammatory[17] activities. Given the easy access to *β*-keto esters,[18] a two-step procedure[19] (Table 2) was deemed suitable for the solid-phase synthesis of dihydropyrano[2,3-*c*]pyrazoles, conveniently introducing various substituents at the 3- and 4-positions (R^1 and R^2). Hydrazine-functionalized photolabile support **4** reacted smoothly with appropriate *β*-keto esters to produce 1*H*-pyrazol-5(4*H*)-one derivatives **9**, which undergo a base-catalyzed three-component reaction with an aldehyde and malononitrile, entailing a tandem Michael addition/Thorpe–Ziegler reaction followed by tautomerization to the dihydropyrano[2,3-*c*]pyrazole **10**. Gratifyingly, the steps of synthesis and photolytic release of the dihydropyrano[2,3-*c*]pyrazoles (**11a–h**) were very clean (Table 2).

In summary, we have developed a photolabile hydrazine linker for the synthesis of peptide hydrazides on a solid support. The synthesis strategy shows excellent compatibility with peptide composition, notably the 20 common proteinogenic *α*-amino acid residues (including side-chain protected analogs) were accepted at the C-terminal hydrazide moiety. The linker is compatible with most commonly used protecting groups for SPPS and remains intact throughout multistep peptide synthesis. Products are ultimately released as hydrazides (*in situ* trapped as acetone-derived hydrazone derivatives) from the solid support in high purity using light. In addition, we have demonstrated the use of the linker for the generation of pharmacologically relevant heterocycles in excellent purity. Finally, we showed the potential of ligating crude deprotected peptide hydrazides to Cys-functionalized peptides in high purity and excellent yield. We expect that this one-pot strategy will enable the synthesis of complex and structurally diverse peptides in the future.

Table 2. Synthesis and Photolytic Release of Dihydropyrano[2,3-*c*]pyrazoles (11a–h)



product	purity[a]	yield	product	purity[a]	yield
 11a	>95%	65%	 11e	95 %	55%
 11b	>95%	62%	 11f	>95%	62%
 11c	91%	43%	 11g	>95%	57%
 11d	95%	67%	 11h	>95%	68%

[a]Purity determined by UPLC.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and full spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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