Chemistry–A European Journal
Research Article
doi.org/10.1002/chem.202201121
Chemistry Europe
European Chemical Societies Publishing
www.chemeurj.org

Solid-Phase Peptide Modification via Deaminative Photochemical Csp^3-Csp^3 Bond Formation Using Katritzky Salts

Joseph Openy,[a] Gulshan Amrahova,[a] Jen-Yao Chang,[a] Anaïs Noisier,[b] and Peter 't Hart*[a]

Abstract: Introduction of unnatural amino acids can significantly improve the binding affinity and stability of peptides. Commercial availability of such amino acids is limited, and their synthesis is a long and tedious process. We here describe a method that allows the functionalization of peptides directly on solid-support by converting lysine residues to Katritzky salts, and subjecting them to a photochemical Giese reaction under mild reaction conditions. The method avoids the need for amino acid synthesis and instead offers a late-stage modification route for rapid peptide diversification. While numerous modification approaches at the lysine amine have been described, this work provides the first example of deaminative functionalization of peptides at lysine. The two-step protocol is compatible with various substrates, lysine analogues, resins, and all proteinogenic amino acids. Finally, by leveraging solid-phase modification, this protocol facilitates the functionalization of longer peptides as was demonstrated using biologically relevant peptides of up to 15 amino acids.

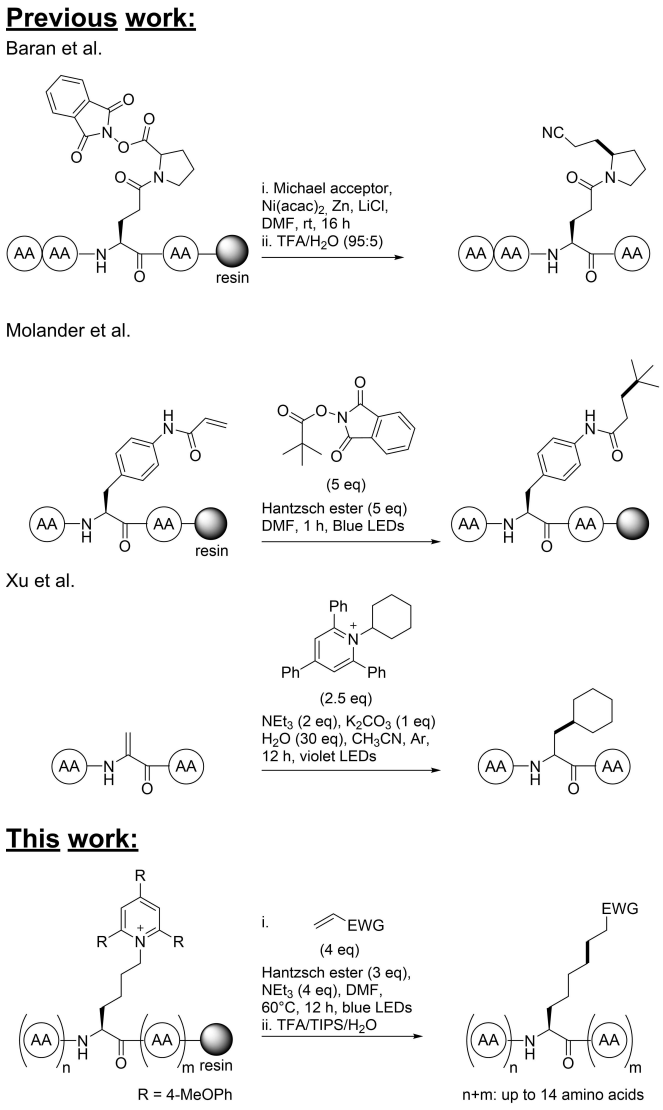
Introduction

Peptides are highly valuable both in drug discovery and as tools in biological research.[1] Solid-phase peptide synthesis (SPPS) has facilitated easy preparation of longer sequences, making synthetic peptides readily accessible.[2] The properties of a peptide, such as affinity towards a biological target and stability against proteases, can often be improved by the introduction of unnatural amino acids.[3] However, commercially available non-proteinogenic amino acids tend to be expensive and diversity is limited which restricts the straightforward modification of a peptide scaffold. Unnatural amino acids that are not commercially available can be synthesized before incorporation into the peptide sequence, but these multistep procedures are time and resource consuming, and it can be difficult to obtain amino acids in good optical purity at the α-carbon. Therefore, numerous methods for the late-stage functionalisation (LSF) of peptides are currently being developed.[4] While many reports focus on the LSF of semi-protected or unprotected peptides in solution, modifications introduced during the solid-phase stage facilitate purification and allow functionalisation of longer peptides, which are often insoluble in organic solvents. Csp^3-Csp^3 bond forming reactions are especially promising for the rapid diversification of amino acids, providing structurally diverse side chains with interesting medicinal chemistry properties. However, most conditions for such reactions are not compatible with SPPS (i.e. incompatible solvents, use of inorganic salts, or high temperatures).[5] A solid-phase method for modifying side chains of peptides was reported by the Baran group who used redox active esters derived from aspartic acid or glutamic acid as radical precursors, followed by nickel catalysed Csp^3-Csp^3 bond formation using dialkylzinc as nucleophiles.[6] This elegant method allows modification of peptides on solid-support but the use of dialkylzinc reagents limits the throughput and structural diversity that can be introduced. The same group adapted the decarboxylative method by applying Giese type reactions to functionalize terminal proline residues on solid-phase (Scheme 1).[7] The MacMillan group applied a similar Giese reaction via an iridium catalysed decarboxylative reaction to perform peptide cyclization and later showed that this chemistry was compatible with *C*-terminal peptide and protein modification.[8] Also using iridium catalysed chemistry, the Jiu group demonstrated the Giese modification of dehydroalanine containing peptides using C–H activation to produce aminoalkyl radicals.[9] More recently the group of Molander described a light-mediated reaction between external redox active esters and Michael acceptor-modified peptides on resin (Scheme 1).[10] The method avoids the use of metal catalysts but uses the Hantzsch ester instead to form an electron-donor/acceptor (EDA) complex that after irradiation with blue light leads to decarboxylative radical formation of the redox active ester. Through these reports, both

[a] *J. Openy, G. Amrahova, J.-Y. Chang, Dr. P. 't Hart*
Chemical Genomics Centre of the Max Planck Society
Max Planck Institute of Molecular Physiology
Otto-Hahn-Strasse 11, 44227 Dortmund (Germany)
E-mail: peter.t-hart@mpi-dortmund.mpg.de

[b] *Dr. A. Noisier*
Medicinal Chemistry, Research and Early Development
Cardiovascular, Renal and Metabolism
BioPharmaceutical R&D, AstraZeneca
Gothenburg (Sweden)

Supporting information for this article is available on the WWW under https://doi.org/10.1002/chem.202201121



Scheme 1. Methods to introduce amino acid side chains at late stage.

groups demonstrated that the Giese reaction is compatible with peptides anchored on solid supports.

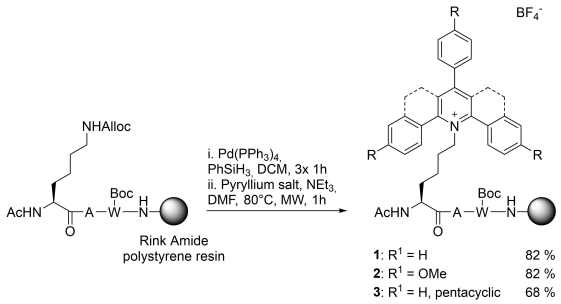
In our search for reactions that could be applied during peptide solid-phase synthesis, our attention was drawn to deaminative C–C bond forming reactions using Katritzky salts.[11] A Katritzky salt is formed from a primary amine and a precursor pyrylium salt and allows efficient homolytic cleavage of the C–N bond.[12] The required amines are abundant in peptides, with the side chain of lysine residues (as well as its shorter analogues) and the *N*-terminus providing ample opportunity for modification. We were especially attracted to photochemical reactions since the conditions are very mild and the typical solvents used are suitable for SPPS (i.e.: DMF, DMA, NMP).[11a,13] Furthermore, the soluble photochemical reductants are a good alternative to the use of insoluble manganese or zinc. These powders are commonly used for reductive cleavage of Katritzky salt C–N bonds and are inseparable from the resin, complicating potential further reaction steps.[14] Interestingly, the report by Molander is one of the few demonstrating photochemistry to be compatible with SPPS.[4g,10] Various reports have recently demonstrated the use of Katritzky salts as alternative to redox active esters for the functionalization of peptides, including a report by the group of Lou who used external Katritzky salts to modify dehydroalanine containing peptides (Scheme 1).[13f,15] In contrast, the group of Liu prepared Katritzky salts from the *N*-terminus of amino acids and dipeptides and reacted them with 2-isocyanobiphenyl under photochemical conditions to form 6-substituted phenanthridines.[15b] These methods demonstrated that photochemical conversion of peptides using Katritzky salts was possible in solution phase but are limited to short sequences (2-6 amino acids) and yield products as diastereomeric mixtures. A few methods also use external Katritzky salts to selectively modify tryptophan residues on peptides and proteins.[16] However, although lysine features among the most commonly modified amino acids; owing to the nucleophilic properties of its side chain amine; to our knowledge the deaminative functionalization of peptides at the lysine residue is yet to be described. We envisioned that unlike the use of external Katritzky salts (which requires synthesis of every substrate prior to peptide functionalization), the introduction of the radical precursor on the peptide, would provide an effective strategy for its rapid diversification with an array of commercially available Michael acceptors. Only the peptide would undergo the Katritzky salt formation, and from a single batch of starting material, many new analogues could be quickly generated. Furthermore, by performing the installation of the Katritzky salts on the side chains of lysine directly on solid-support, only operationally simple steps would be required. Nonetheless, this approach could be more challenging as less stable primary radicals would be formed on resin prior to reaction with radical traps in the solution phase.

Here we describe a photochemical method for the functionalization of lysine-containing peptides via a deaminative Giese-type Csp^3-Csp^3 bond forming reaction on solid-phase. The method comprises two steps, first the conversion of the Lys to a Katritzky salt followed by EDA complex mediated deaminative alkylation with Michael acceptor substrates (Scheme 1). Katritzky salts were installed by reacting the free ε-amine of Lys-containing peptides on solid-support with pyrylium salts. After successful incorporation of the Katritzky salt, the reaction conditions for the photochemical Giese reaction on solid-phase were optimized. Using these mild and metal-free reaction conditions a variety of Michael acceptors were tested to demonstrate the substrate scope. The efficiency of the reaction with non-proteinogenic Lys-analogues as well as the peptide *N*-terminus was also explored. The compatibility of the reaction with various linkers and resins commonly employed in SPPS was evaluated. Next, model peptides were used to quickly assess the tolerance of the reaction to the presence of all 20 proteinogenic amino acids. Finally, to demonstrate the usefulness of this novel methodology several biologically relevant peptides were selectively functionalized.

Results and Discussion

While the Ni-catalyzed deaminative arylation of the side chain of lysine was previously reported by Watson et al.,[11b] we deemed these conditions unsuitable for SPPS since it required solvents that are not compatible with standard peptide resins. Furthermore, the strong basic conditions (i.e., KOtBu) led to racemization at the lysine α-carbon. Instead, we were drawn to the conditions described by the group of Aggarwal who used the Hantzsch ester to form an EDA complex with the Katritzky salt. Irradiation with blue light initiates photoinduced electron transfer (PET) which in turn causes homolytic C–N bond cleavage.[13h] The formed carbon radical can engage in a Csp^3-Csp^3 bond formation with a Michael acceptor which then undergoes a hydrogen atom transfer (HAT) from the Hantzsch ester to form the final product. The use of moisture and air stable reagents, mild conditions, and solvents compatible with solid-support made this methodology an attractive choice for the solid-phase modification of peptides. This report presented a rare example of a Giese-type reaction using non-stabilized primary alkyl radicals. However, Lys was not among the highlighted substrates and since lower yields were obtained slight modifications of the initial reaction conditions were required.

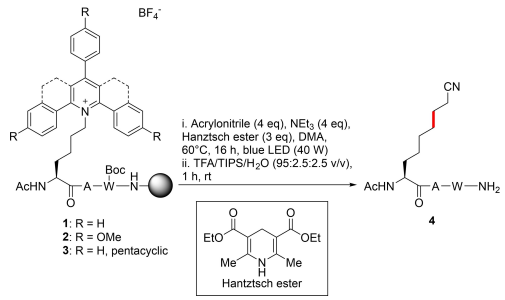
To evaluate the feasibility of both the formation of lysine derived Katritzky salts and the subsequent photochemically induced deamination reaction on resin, we designed and synthesized a short resin-bound tripeptide substrate (Scheme 2). The tripeptide contains the lysine to be modified at the *N*-terminus, an alanine in position 2 providing some spacing and a tryptophan at the *C*-terminus in order to facilitate UV detection for HPLC analysis and purification. The tripeptide was synthesized on Rink Amide polystyrene resin, the *N*-terminus was acetylated, and the N_ε-Alloc group was removed using $Pd(PPh_3)_4$ in the presence of $PhSiH_3$ to yield the free amine. Preparation of the Katritzky salts is a straightforward condensation between the amine and a pyrylium salt (typically 2,4,6-triphenylpyrylium tetra-fluoroborate).[11b,12] Traditionally, ethanol is used as the solvent for this reaction, but we chose to use DMF instead for its superior resin swelling properties. The pyrylium salt was used in a small excess (1.5 equivalents) and the reaction heated in a microwave at 80 °C for one hour in the presence of triethylamine, yielding the desired product **1** in 82% isolated yield. To determine the yield a portion of the resin was cleaved and deprotected using concentrated TFA and the product purified by normal phase chromatography. Variations of the Katritzky salt have been described and previous results have shown that the commonly used unsubstituted variant **1** is not optimal for reactions where a primary radical is formed.[14a] Therefore, we also prepared the 4-methoxy substituted Katritzky salt **2** as well as the pentacyclic salt **3** since both have been reported as more reactive during C–N bond cleavage (Scheme 2).[12,14a,17] The pyrylium salts were prepared according to previously described methods and installed using the same conditions as for compound **1**.[17,18] Compounds **2** and **3** were also obtained in good yields of 81% and 68%.



Scheme 2. Synthesis of the model tripeptide with various Katritzky salt modifications. Yields determined after cleavage using TFA/TIPS/H_2O (95 : 2.5 : 2.5 v/v, 1 h) and silica flash chromatography purification.

Next, we tested the Giese reaction following a protocol previously described by Aggarwal et al.[13h] Reduced diffusion of reagents during solid-phase chemistry often makes higher reagent loading a requirement for good conversion. Therefore, we adapted the reaction conditions by increasing the amount of triethylamine (to 4 equivalents) and Michael acceptor (to 4 equivalents). Owing to the nature of the solid-phase reaction, it was not possible to use internal standards to determine the yields of the reactions quickly and accurately prior to the purification step. We therefore opted to only report isolated yields obtained after both cleavage and preparative RP-HPLC purification of the products, as it also reflects the yields one would obtain when applying the method to biologically active peptides. We used the previously determined yields of the purified Katritzky salt peptides to calculate resin-loading before the Giese reaction. We chose acrylonitrile as the Michael acceptor for our initial attempts and ran the reaction at 60 °C for 16 h while irradiating with a 40 W blue LED Kessil lamp. Of all three Katritzky salts, the methoxy substituted variant **2** was significantly superior, providing compound **4** with a yield of 59% compared to only 6% and 13% for salt **1** and **3**, respectively (Table 1, entries 1–3). This result is in line with the previous report describing the higher yields for the methoxy substituted Katritzky salts in reactions where primary radicals are formed.[14a] Reducing the amount of Michael acceptor to 2.6 equiv. reduced the yield accordingly (Table 1, entry 4) and exclusion of any of the reaction components led to complete loss or only trace amounts of product (Table 1, entries 5–8). A reduction of the reaction time to 1 h also led to only traces of product being formed (Table 1, entry 9) and when non-dried and non-purged solvent was used the reaction proceeded but with a diminished yield of 26% (Table 1, entry 10). With suitable reaction conditions in hand, a variety of Michael acceptors was used to explore the scope of the reaction (Scheme 3). All yields for these products were determined after TFA cleavage and reverse phase HPLC purification. Besides nitrile derivative **4**, various other modifications were obtained in good yields including sulfone **5**, 2-pyridine **6**, pyrazine **7** and biphenyl **9**. The methyl and benzyl esters **11** and **12** worked well while the tert-butyl acrylate derived **10** was obtained in low yield. Interestingly, the reaction with benzylacrylate **12** provided a higher yield than was previously described by Aggarwal et al. in a similar reaction with an unsubstituted Katritzky salt derived

Table 1. Optimization of the conditions for the solid-phase photoinduced Giese reaction.

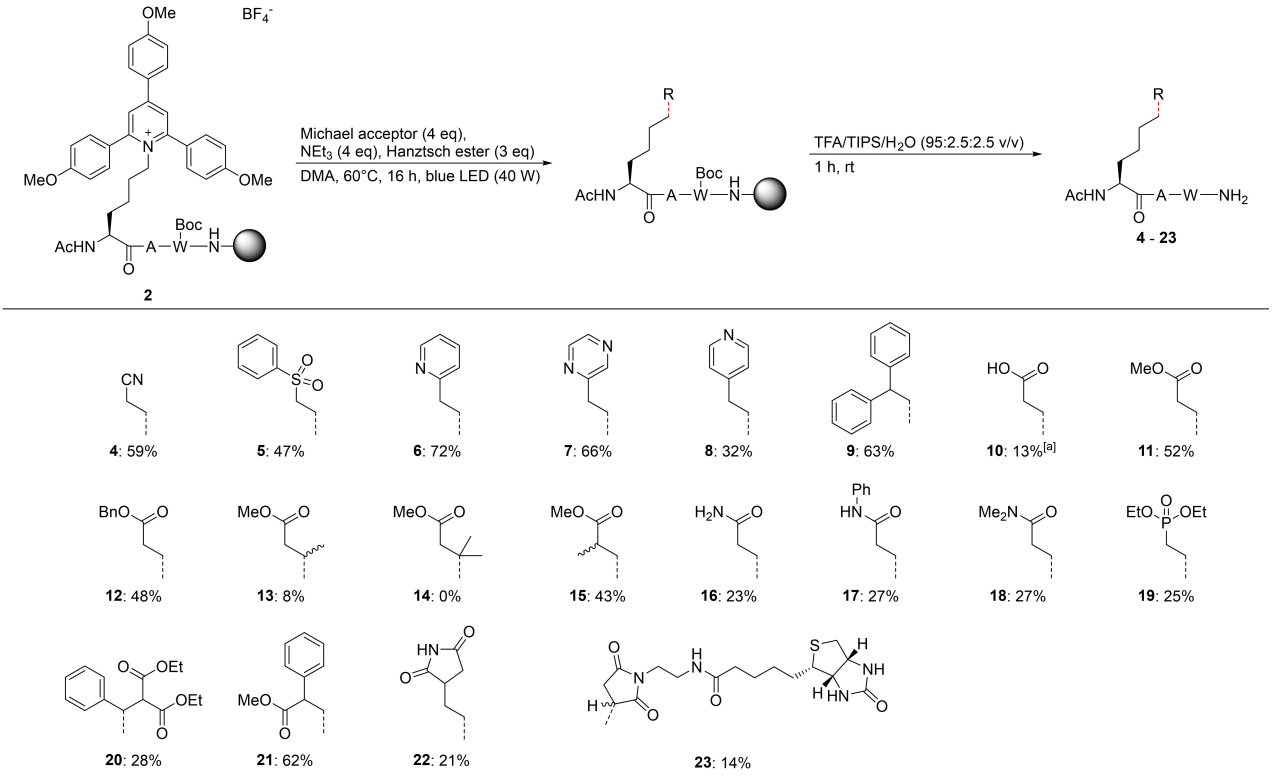


Entry	Starting material	Modification[a]	Yield of **4** [%][b]
1	**1**	–	6
2	**2**	–	59
3	**3**	–	13
4	**2**	2.6 equiv. Michael acceptor	22
5	**2**	No LED	0
6	**2**	No Hantzsch ester	trace
7	**2**	No NEt_3	trace
8	**2**	Room temperature	trace
9	**2**	Reaction time: 1 h	trace
10	**2**	No precautions[c]	26

[a] All modifications were made in the first reaction step. [b] Yields were determined after both reaction steps and reverse phase HPLC purification. [c] Solvent was neither dried nor degassed.

primary radical, indicating that the methoxy substituted Katritzky salt is indeed more suitable for such substrates.[13h,14a]

More sterically congested variants of methyl ester **11** resulted in poor yield or no product formation (compounds **13** and **14**) but an extra methyl group in the α-position was tolerated (compound **15**). The bulkier diester **20** gave a lower yield, but the monoester **21** performed very well. Various amides performed with similar efficiencies (compounds **16**–**18**) as well as phosphonate **19** which can serve as a phosphorylated amino acid mimic. Although compounds **13**, **15**, and **20** are expected to provide diastereomers, none could be observed by either NMR or HPLC. It is possible that the long flexible carbon chain between the chiral centre and the peptide prevents easy discrimination of the isomers by either technique. Compound **22** was successfully formed by using maleimide which prompted us to investigate maleimide functionalized biotin to form compound **23**. The successful installation of the biotin handle demonstrates the applicability of the reaction in the preparation of biological tool compounds. Except for the carboxylic acid **10** and its ester derivatives, all the described modifications cannot be obtained as amino acids from commercial sources. Such modifications would require multi-step synthesis including various protecting group manipulations which are time and resource consuming. Although the yields were determined after preparative HPLC purification, which is often a cause for some product loss, we analysed the reactions further for the formation of side products. LC–MS analysis indicated that the lower reactivity of some of the Michael acceptors led to premature HAT to the primary radical generated after homolytic cleavage of the C–N bond. To test

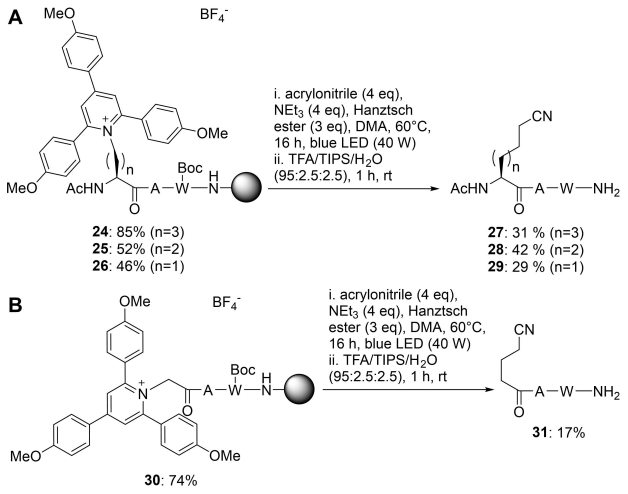


Scheme 3. Scope of Michael acceptors used in the photochemical solid-phase Giese reaction. Isolated yields were corrected based on the isolated yields of the Katritzky salt peptide. [a] tert-butyl acrylate was used in the Giese reaction.

whether yields could be improved for compound **10** we increased the Michael acceptor to 8 eq, but LCMS analysis of the cleaved product indicated reduced product formation in this case due to a second addition of the Michael acceptor (see Supporting Information Figure S2). From this we concluded that the reaction conditions were already optimal as there is a balance between the formed side products.

Having evaluated the scope of Michael acceptors, we turned to the exploration of various peptide substrates. To probe whether lysine analogues with shorter side chains could also be derivatized we synthesized a series of peptide substrates with Alloc protected ornithine, diaminobutyric acid, and diaminopropionic acid (Scheme 4A). The peptides were modified using the same conditions for Alloc deprotection, Katritzky salt formation (Scheme 4A, compounds **24**–**26**) and photochemical Giese reaction (compounds **27**–**29**). The efficiency of the Katritzky salt formation showed a clear correlation with the length of the side chain. Yields were the highest for the four (compound **2**) and three carbon chains (compound **24**) and lower but still acceptable for the two and one carbon variants. It is likely that steric factors reduce the reactivity of the shorter side chain analogues. For all peptides, the Giese reaction was performed using acrylonitrile as the Michael acceptor. The yields were good albeit lower than that of the lysine peptide **4**. Interestingly, for the Giese reaction, no trend was found between the length of the side chains and the reaction efficiency. The diaminopropionic acid containing peptide **29** was analyzed by NMR for possible epimerization at the α position but only a single diastereomer was observed indicating that the chiral centre remained intact under the reaction conditions. Unlike the N_ε of lysine, the *N*-terminus of amino acids and peptides have already been subjected to deaminative reaction using Katritzky salts by other research groups. In 2017, Glorius et al. reported the photocatalytic deaminative Minisci reaction of amino acid feedstocks in solution while the deaminative intramolecular Giese cyclisation was described by Xu et al. using ionic compound-promoted cleavage of the Katritzky salt.[11a,15d] To demonstrate the applicability of our methodology for the Giese reaction at the *N*-terminus of resin-bound peptide substrates, we synthesized a model tripeptide where the *N*-terminal lysine residue was replaced with glycine (to avoid diastereoisomer formation) and converted the *N*-terminus primary amine to the Katritzky salt **30** with a good yield of 74% (Scheme 4B). The Giese reaction was performed with acrylonitrile leading to 17% product formation. In this case the reduced reactivity could be explained by the decreased nucleophilicity of the radical formed at the α position.

Peptide chemists can rely on a collection of available resins and linkers for SPPS, as the choice of both the spacer and solid-support can deeply impact the overall performance of the synthesis and should therefore be adapted to the targeted amino acid sequences. Therefore, we decided to probe a selection of different linkers and resins and compare the reaction efficiency to that of the Rink Amide polystyrene (PS) resin we had been using until now (Table 2). Rink Amide ChemMatrix resin was chosen to explore the effect of a different polymer (PEG), while the PS Wang and 2-chlorotrityl chloride (2-CTC) resins were used to verify the compatibility of various linkers with the reaction conditions. Both Wang and 2-CTC linkers provide peptides with *C*-terminal carbox-ylic acids, but the highly acid sensitive 2-CTC also allows cleavage of peptides from the resin without simultaneous deprotection of the side chain protecting groups. After synthesis of our model tripeptide on the selected linker-resins, we performed the Katritzky salt formation and Giese reaction using 2-vinylpyridine as the Michael acceptor. All resins were found to be compatible with Katritzky salt formation except for the PS 2-CTC resin. We therefore replaced it with the PS HMPB resin since it also provides *C*-terminal carboxylic acids and is cleavable under low acid concentrations. All resin-bound Katritzky salt peptides, except for PS 2-CTC **34**, were then tested in the photochemical Giese reaction and were successfully functionalized (Table 2). The poor yield observed for the ChemMatrix resin was caused by the additional quantity of solvent required to accommodate the high swelling properties of the PEG based polymer, thus diluting the reaction mixture. The dilution led to a significant amount of side product formed through premature HAT.



Scheme 4. A) Katritzky salt formation and Giese reaction on resin-bound peptide substrates with lysine analogues of various side chain lengths. B) Giese reaction at the *N*-terminus of resin-bound peptide substrate.

Table 2. Compatibility of various linkers and resin polymers with Katritzky salt formation and Giese reaction.

Linker	Resin	Loading [mmol/g]	Nr. (Kat. salt)	Yield [%]	Nr. (Giese prod.)	Yield [%][a]
Rink Amide	PS	0.63	**2**	81	**6**	72
Rink Amide	PEG[b]	0.40	**32**	53	**36**	5
Wang	PS	0.45	**33**	49	**37**	21
2-CTC	PS	0.79	**34**	0	-	-
HMPB	PS	0.50	**35**	70	**38**[c]	32
HMPB	PS	0.50	**35**	70	**39**[d]	29

[a] Yields were determined after both reaction steps and reverse phase HPLC purification. [b] ChemMatrix resin [c] 2-vinylpyridine was used as Michael acceptor. [d] acrylonitrile was used as Michael acceptor.

Although the highest yield was obtained with the Rink Amide polystyrene resin, these results demonstrate that other linkers and resins are also suitable for peptide modification via the photochemical Giese reaction. Since the HMPB resin performed well, we also tested the Giese reaction using acrylonitrile and obtained a yield of 29% (compound **39**).

Next, a set of 5 model hexapeptides was synthesized on Rink Amide polystyrene resin. A lysine residue was inserted in the third position and a tryptophan at the *C*-terminus to facilitate UV detection while the other 4 positions were randomized to cover the remaining proteinogenic amino acids (Table 3). The peptides were all subjected to the same synthetic steps of Alloc removal, Katritzky salt formation, photochemical Giese reaction and TFA cleavage. The desired products were obtained for all 5 peptides although the preparative HPLC purification had a significant effect on the yields. It is important to note that in contrast to the previous experiments, we calculated the yields for these peptides over the entire synthesis of 18 reaction steps starting from resin loading. These results indicate that all amino acids and side chain protecting groups commonly employed in Fmoc SPPS are compatible with the reaction conditions, affording modified peptides in yields commonly observed in peptide synthesis.

The poor solubility of longer peptides in organic solvents and the lack of protocols compatible with aqueous media, hampers the late-stage modification of complex peptides using solution phase chemistry. The development of methodologies for the LSF of peptides directly on solid-support provides an interesting alternative. To demonstrate the applicability of our on-resin photochemical method to the diversification of longer and biologically active peptides, we chose a histone 3 tail peptide of 10 amino acids and a p53 derived peptide containing 15 amino acids (Figure 1).[19] Both peptides were synthesized on Rink Amide polystyrene resin and taken through the steps of Alloc deprotection and Katritzky salt formation. After splitting the resin into equal portions, two different Michael acceptors were used in the Giese reaction to produce the modified histone peptides **44** and **45** in yields of 13 and 19% respectively (Figure 1, yields were calculated over the entire synthetic routes starting from resin loading). As a comparison the unmodified peptide **46** was prepared in 48% yield. Similarly compounds **47** and **48** resulting from the longer p53 peptide were isolated in 9% and 13% yields while the unmodified peptide **49** was obtained in 25% yield (Figure 1). These results showcase the operational simplicity and efficiency of LSF of peptides on solid-support. As from a single batch of starting material, various analogues were readily produced just by splitting the resin.

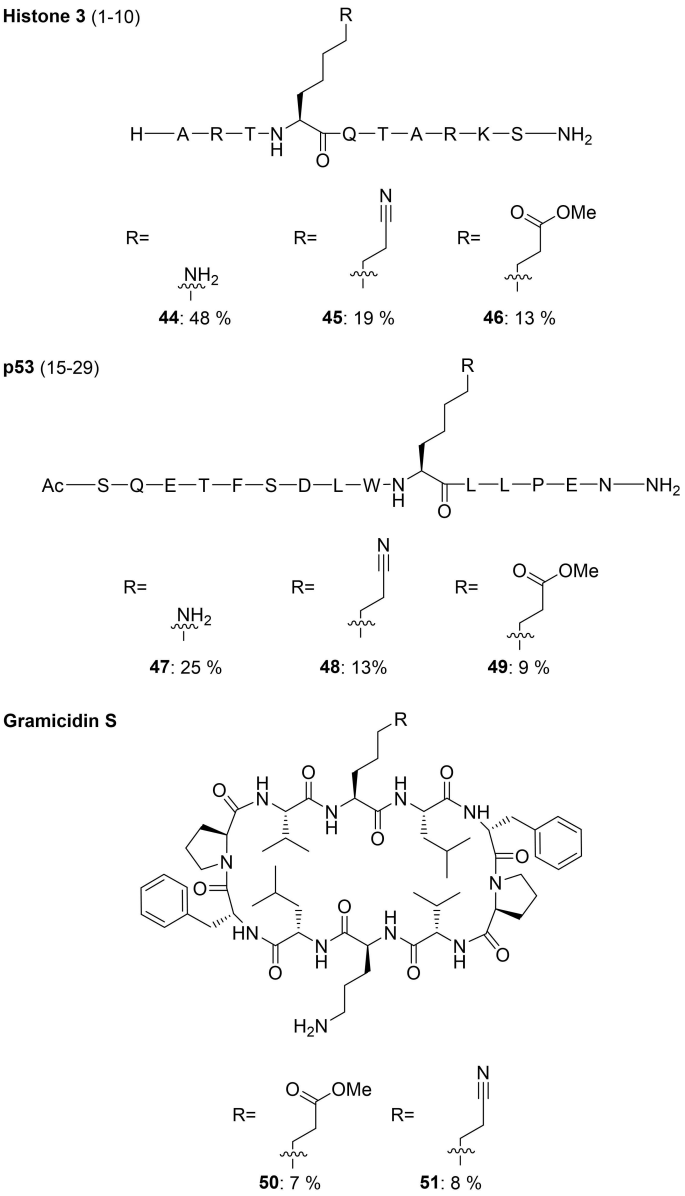
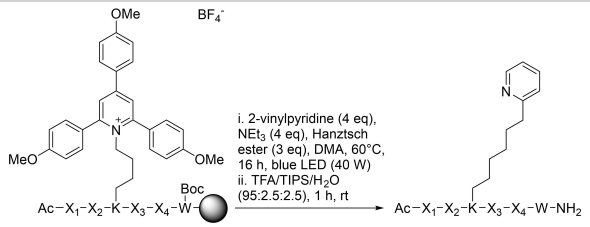


Figure 1. Synthesized biologically active peptides with modifications.

Table 3. Compatibility of all 20 proteinogenic amino acids with the Katritzky salt formation and Giese reaction.



Compound nr.	X_1	X_2	X_3	X_4	Yield [%][a]
39	His(Trt)	Gly	Ala	Asp(tBu)	4
40	Val	Thr(tBu)	Arg(Pbf)	Met	13
41	Ile	Gln(Trt)	Pro	Phe	8
42	Tyr(tBu)	Ser(tBu)	Gly	Leu	13
43	Asn(Trt)	Cys(Trt)	Lys(Boc)	Glu(tBu)	9

[a] Yields were determined over all steps starting from resin loading.

After successful modification of linear peptides, we decided to attempt the functionalization of more complex substrates such as the cyclic peptide antibiotic Gramicidin S which contains two ornithine residues.[20] Modification of these ornithines can lead to antimicrobially active compounds with reduced haemolytic properties.[21] A solid-phase synthesis on

Wang resin has been previously reported and was adapted to include the Katritzky salt formation and Giese reaction at one of the ornithine residues (see Figure 1 and Supporting Information Scheme S1).[22] One of the ornithine residues was protected with the Alloc group while the other was protected with the orthogonal ivDde group. After SPPS, the Alloc group was removed using the standard Pd(0) conditions followed by reaction with the pyrylium salt to form the Katritzky modified ornithine. The Giese reaction was then performed with either methyl acrylate or acrylonitrile and followed by cleavage of the peptide from the resin, cyclization in solution, and removal of the ivDde group. The desired peptides **50** and **51** containing a single modified ornithine were obtained in 7% and 8% yields over the entire synthesis including reverse phase HPLC purification. As already demonstrated for peptides **48** and **49**, the use of the easily removable Alloc as an orthogonal protecting group in our solid-phase LSF strategy facilitates the selective modification of a single amine side chain. Furthermore, in peptides **50** and **51**, the introduction of ivDde as an additional orthogonal protecting group allows further selective modification of a side chain amine following the Giese reaction to take place.

Conclusion

The introduction of non-natural amino acids in a peptide is traditionally done by synthesizing amino acid building blocks followed by incorporation during solid-phase peptide synthesis. Asymmetric synthesis of amino acids is a long and tedious process with many synthetic steps, low yields, and challenging control of the stereochemistry at the α-carbon. We have shown here that amino acid residues can effectively be modified directly during the solid-phase stage. By using primary amines as starting points and converting them to Katritzky salts prior to a subsequent photochemical Giese reaction with commercially available Michael acceptors, a diverse range of non-natural analogues could be quickly accessed. This work provides the first example of deaminative functionalization of peptides at the lysine residue and its shorter analogues. Furthermore, both photochemistry and Csp^3-Csp^3 bond formation on solid-phase are rarely described but are easily facilitated by the described method. As demonstrated, the strategy allows for the site-selective late-stage modification of longer and more complex peptides and represents a novel conjugation approach. Rapid diversification via this strategy allows access to many peptide derivatives for structure-activity relationship analysis and property optimization. The method described here also opens avenues for further exploration of peptide Katritzky salts for introduction of an even larger variety of unnatural side chains.

Experimental Section

General method for peptide Katritzky salt formation: A 10 mL microwave vial containing dry peptidyl resin (300 μmol) was charged with a solution of pyrylium salt (1.5 equiv.) and NEt_3 (4 equiv.) in DMF (4 ml) and the reaction mixture was heated for 1 h at 80 °C under microwave irradiation. Additional DMF was added if necessary to completely suspend the resin. The peptidyl resin was filtered and washed with DMF (4×1 min), CH_2Cl_2 (2×1 min), and Et_2O (1×1 min) then dried under high vacuum.

General method for peptide Giese reaction: A flame dried reaction vial with a magnetic stirring bar under argon atmosphere was charged with peptidyl resin (100 μmol) and sealed. The vial was then charged with a solution of Hantzsch ester (76.0 mg; 300 μmol; 3.00 equiv.) in DMA (400 μl) followed by three evacuation-backfill cycles with argon and thereafter, charged with a solution of Michael acceptor (4 equiv.) and NEt_3 (4 equiv.) in DMA (300 μl). Additional DMA was added if necessary to completely suspend the resin. The vial was affixed in a 100 mL beaker that was then sealed with aluminum foil. The beaker was placed in an oil bath with the temperature set to 100 °C followed by irradiation with blue LEDs. The combined heat of the lamp and oil bath maintained an internal temperature of 65 °C in the beaker (see Supporting Information Figure S1). After 16 h, the peptidyl resin was filtered and washed with DMF (4×1 min) and CH_2Cl_2 (2×1 min).

Acknowledgements

We kindly acknowledge H. Waldmann, C. Loh, and L. Knerr for critical discussions regarding the manuscript. Christiane Heitbrink is kindly acknowledged for HRMS measurements. G.A. was funded by a scholarship from the German Academic Exchange Service (DAAD). J.-Y. C. was funded by a Government Scholarship for Overseas Study by the Ministry of Education, Taiwan. Open Access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: Giese reaction · late-stage functionalization · peptides · peptidomimetics · photochemistry

[1] a) D. J. Drucker, *Nat. Rev. Drug Discovery* **2020**, *19*, 277–289; b) A. A. Vinogradov, Y. Yin, H. Suga, *J. Am. Chem. Soc.* **2019**, *141*, 4167–4181; c) A. Zorzi, K. Deyle, C. Heinis, *Curr. Opin. Chem. Biol.* **2017**, *38*, 24–29; d) A. Henninot, J. C. Collins, J. M. Nuss, *J. Med. Chem.* **2018**, *61*, 1382–1414.
[2] a) B. Merrifield, *Science* **1986**, *232*, 341–347; b) I. Coin, M. Beyermann, M. Bienert, *Nat. Protoc.* **2007**, *2*, 3247–3256.
[3] a) Y. Ding, J. P. Ting, J. Liu, S. Al-Azzam, P. Pandya, S. Afshar, *Amino Acids* **2020**, *52*, 1207–1226; b) Y. V. Guillen Schlippe, M. C. T. Hartman, K. Josephson, J. W. Szostak, *J. Am. Chem. Soc.* **2012**, *134*, 10469–10477; c) L. J. Walport, R. Obexer, H. Suga, *Curr. Opin. Biotechnol.* **2017**, *48*, 242–250; d) M. Muttenthaler, G. F. King, D. J. Adams, P. F. Alewood, *Nat. Rev. Drug Discovery* **2021**, *20*, 309–325; e) D. J. Craik, M.-W. Kan, *Expert Opin. Drug Discovery* **2021**, *16*, 1399–1402.
[4] a) A. F. M. Noisier, M. A. Brimble, *Chem. Rev.* **2014**, *114*, 8775–8806; b) S. Sengupta, G. Mehta, *Tetrahedron Lett.* **2017**, *58*, 1357–1372; c) W. Wang, M. M. Lorion, J. Shah, A. R. Kapdi, L. Ackermann, *Angew. Chem. Int. Ed.*

2018, *57*, 14700–14717; *Angew. Chem.* **2018**, *130*, 14912–14930; d) L. R. Malins, *Curr. Opin. Chem. Biol.* **2018**, *46*, 25–32; e) L. R. Malins, *Pept. Sci.* **2018**, *110*, e24049; f) C. Bottecchia, T. Noël, *Chem. Eur. J.* **2019**, *25*, 26–42; g) T. A. King, J. Mandrup Kandemir, S. J. Walsh, D. R. Spring, *Chem. Soc. Rev.* **2021**, *50*, 39–57.

[5] T. Willemse, W. Schepens, H. Vlijmen, B. Maes, S. Ballet, *Catalysts* **2017**, *7*, 74.

[6] T. Qin, J. Cornella, C. Li, L. R. Malins, J. T. Edwards, S. Kawamura, B. D. Maxwell, M. D. Eastgate, P. S. Baran, *Science* **2016**, *352*, 801–805.

[7] T. Qin, L. R. Malins, J. T. Edwards, R. R. Merchant, A. J. E. Novak, J. Z. Zhong, R. B. Mills, M. Yan, C. Yuan, M. D. Eastgate, P. S. Baran, *Angew. Chem. Int. Ed.* **2017**, *56*, 260–265; *Angew. Chem.* **2016**, *129*, 266–271.

[8] a) S. J. McCarver, J. X. Qiao, J. Carpenter, R. M. Borzilleri, M. A. Poss, M. D. Eastgate, M. M. Miller, D. W. C. MacMillan, *Angew. Chem. Int. Ed.* **2017**, *56*, 728–732; *Angew. Chem.* **2017**, *129*, 746–750; b) S. Bloom, C. Liu, D. K. Kölmel, J. X. Qiao, Y. Zhang, M. A. Poss, W. R. Ewing, D. W. C. MacMillan, *Nat. Chem.* **2018**, *10*, 205–211.

[9] R. A. Aycock, C. J. Pratt, N. T. Jui, *ACS Catal.* **2018**, *8*, 9115–9119.

[10] M. Elkhalifa, M. B. Elbaum, D. M. Chenoweth, G. A. Molander, *Org. Lett.* **2021**, *23*, 8219–8223.

[11] a) F. J. R. Klauck, M. J. James, F. Glorius, *Angew. Chem. Int. Ed.* **2017**, *56*, 12336–12339; *Angew. Chem.* **2017**, *129*, 12505–12509; b) C. H. Basch, J. Liao, J. Xu, J. J. Piane, M. P. Watson, *J. Am. Chem. Soc.* **2017**, *139*, 5313–5316.

[12] A. R. Katritzky, C. M. Marson, *Angew. Chem. Int. Ed.* **1984**, *23*, 420–429; *Angew. Chem.* **1984**, *96*, 403–413.

[13] a) F.-S. He, S. Ye, J. Wu, *ACS Catal.* **2019**, *9*, 8943–8960; b) J. T. M. Correia, V. A. Fernandes, B. T. Matsuo, J. A. C. Delgado, W. C. de Souza, M. W. Paixão, *Chem. Commun.* **2020**, *56*, 503–514; c) J. A. C. Delgado, J. T. M. Correia, E. F. Pissinati, M. W. Paixão, *Org. Lett.* **2021**, 21–25; d) Q. Xia, Y. Li, X. Wang, P. Dai, H. Deng, W. Zhang, *Org. Lett.* **2020**, *22*, 7290–7294; e) K. J. Berger, M. D. Levin, *Org. Biomol. Chem.* **2021**, *19*, 11–36; f) Y. Xu, Z. Xu, Z. Liu, H. Lou, *Org. Chem. Front.* **2019**, *6*, 3902–3905; g) J. Yi, S. O. Badir, L. M. Kammer, M. Ribagorda, G. A. Molander, *Org. Lett.* **2019**, *21*, 3346–3351; h) J. Wu, P. S. Grant, X. Li, A. Noble, V. K. Aggarwal, *Angew. Chem. Int. Ed.* **2019**, *58*, 5697–5701; *Angew. Chem.* **2019**, *131*, 5753–5757.

[14] a) R. Martin-Montero, V. R. Yatham, H. Yin, J. Davies, R. Martin, *Org. Lett.* **2019**, *21*, 2947–2951; b) H. Yue, C. Zhu, L. Shen, Q. Geng, K. J. Hock, T. Yuan, L. Cavallo, M. Rueping, *Chem. Sci.* **2019**, *10*, 4430–4435; c) S. Ni, C.-X. Li, Y. Mao, J. Han, Y. Wang, H. Yan, Y. Pan, *Sci. Adv.* **2019**, *5*, eaaw9516; d) J. Liao, C. H. Basch, M. E. Hoerrner, M. R. Talley, B. P. Boscoe, J. W. Tucker, M. R. Garnsey, M. P. Watson, *Org. Lett.* **2019**, *21*, 2941–2946.

[15] a) I. Kim, H. Im, H. Lee, S. Hong, *Chem. Sci.* **2020**, *11*, 3192–3197; b) Z. F. Zhu, M. M. Zhang, F. Liu, *Org. Biomol. Chem.* **2019**, *17*, 1531–1534; c) C. Wang, R. Qi, H. Xue, Y. Shen, M. Chang, Y. Chen, R. Wang, Z. Xu, *Angew. Chem. Int. Ed.* **2020**, *59*, 7461–7466; *Angew. Chem.* **2020**, *132*, 7531–7536; d) M. Wang, C. Wang, Y. Huo, X. Dang, H. Xue, L. Liu, H. Chai, X. Xie, Z. Li, D. Lu, Z. Xu, *Nat. Commun.* **2021**, *12*, 6873.

[16] a) S. J. Tower, W. J. Hetcher, T. E. Myers, N. J. Kuehl, M. T. Taylor, *J. Am. Chem. Soc.* **2020**, *142*, 9112–9118; b) B. Laroche, X. Tang, G. Archer, R. Di Sanza, P. Melchiorre, *Org. Lett.* **2021**, *23*, 285–289.

[17] a) X. Zeng, W. Yan, S. B. Zacate, A. Cai, Y. Wang, D. Yang, K. Yang, W. Liu, *Angew. Chem. Int. Ed.* **2020**, *59*, 16398–16403; *Angew. Chem.* **2020**, *132*, 16540–16545; b) Y. Ma, Y. Pang, S. Chabbra, E. J. Reijerse, A. Schnegg, J. Niski, M. Leutzsch, J. Cornella, *Chem. Eur. J.* **2020**, *26*, 3738–3743.

[18] K. A. Ogawa, A. E. Goetz, A. J. Boydston, *J. Am. Chem. Soc.* **2015**, *137*, 1400–1403.

[19] a) P. Voigt, D. Reinberg, *ChemBioChem* **2011**, *12*, 236–252; b) G. M. Popowicz, A. Czarna, U. Rothweiler, A. Szwagierczak, M. Krajewski, L. Weber, T. A. Holak, *Cell Cycle* **2007**, *6*, 2386–2392.

[20] G. Nagamurthi, S. Rambhav, *J. Biosci.* **1985**, *7*, 323–329.

[21] K. Yamada, S. S. Shinoda, H. Oku, K. Komagoe, T. Katsu, R. Katakai, *J. Med. Chem.* **2006**, *49*, 7592–7595.

[22] P. Wadhwani, S. Afonin, M. Ieronimo, J. Buerck, A. S. Ulrich, *J. Org. Chem.* **2006**, *71*, 55–61.

Manuscript received: April 12, 2022
Accepted manuscript online: April 19, 2022
Version of record online: May 26, 2022